Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XXXII | *NOVEMBER, 1943* | NUMBER 11 CONSECUTIVE NO. 21

Recent Advances in Synthetic Organic Antisyphilitics*

By W. G. Christiansen and A. E. Juristt

While effective progress has been and is being made in the treatment of syphilis, it is interesting and important to note that the changes that have taken place are not in the fundamentals of syphilis therapy but rather in detail and in doing the job with a better understanding of the aspects involved. Thus, today after some thirty years the arsenicals continue as the foundation on which the treatment of this disease rests, and, what is more, the arsenicals of today are still fundamentally those of Ehrlich. What we have been doing, therefore, is to learn more about how to employ those things that have been at our disposal for a long time, what to use with them, and how to arrange the use of these drugs so that the patient receives a maximum of benefit with a minimum of hazard. This brings home to us the fact that we have not realized on and appreciated the things already available or in hand, and that on a par in importance with the creation of new things is an evaluation and exploitation of what we already have.

It may be best to examine first the situation regarding the antisyphilitic arsenicals themselves. Not many years ago the hydrochloride of 3-amino-4-hydroxy phenyl arsenious oxide,[1] suitably compounded for therapeutic use, began to be employed; first in a small way and then in increasing amounts until today it occupies an important position in this field. Advantages associated with it are that the therapeutic dose in terms of arsenical is only a fraction of what is necessary when the arsphenamine type compounds are used; the effectiveness, even though the dose is small, is good and uniform; and as a logical consequence of the smaller dose and the chemical nature of the compound,[2] the incidence of reactions in the patients, particularly the serious reactions, is low. In addition this preparation enjoys the same practical advantage of convenience that neoarsphenamine and sulfarsphenamine do in comparison with arsphenamine, namely, easy solubility in small volumes of water to yield a solution ready for injection without *p*H adjustment. More recently this same arsenical has come

* This is the second of a series of review articles.

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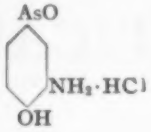
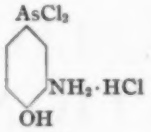
[1] A compound known for a long time by the more convenient name "arsenoxide."

[2] "Arsenoxide" yields a noncolloidal solution, whereas the arsphenamines are to varying degrees colloidal. This is a difference of much significance, because it appears an advantage from the therapeutic standpoint, apparently being related to their low clinical toxicity.

into use in the form of its dichlor derivative compounded in one way or another. The structural formulas for the oxide (I) and the dichloride (II) make the relationship of the products clear.



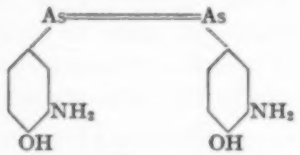
I



II

The materials with which these arsenicals are mixed in the formulating of the therapeutic preparation include substances (alkali carbonate, citrate, etc.) which at the moment of solution neutralize the acidity associated with the hydrochloride grouping that exists in both cases and the dichlorarsine present in the second. The solutions of the dichlorarsine hydrochloride as prepared for injection are essentially the same as those of the oxide hydrochloride in so far as the chemical nature of the arsenical is concerned. Consequently, the advantages of small dosage, effectiveness and low incidence of reactions mentioned above, reside here too. There are things in favor of the dichlorarsine hydrochloride form; it is a more satisfactory chemical compound to work with and is not as sensitive to deteriorating influences as the oxide. Those are differences that can reflect themselves in the products as therapeutic agents and can react to the benefit of the patients receiving them.

A comparison of the structural formula of the oxide (or the dichloride) with that of the arsphenamines reveals a fundamental similarity. Inasmuch as neoarsphenamine and sulfarsphenamine are merely special forms of solubilized arsphenamine, it will suffice for the present purposes to use the basic arsphenamine formula (III). Obviously,



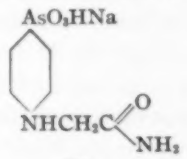
III

both I and II are trivalent arsenicals, both contain the same aromatic nucleus, and the only difference is in the state of the arsenic. The oxide is the first stage of oxidation on the arseno group in the arsphenamine structure, and, conversely, the latter is the product obtained from the former by reduction. Chemically these newer antisyphilitic arsenicals are closely related to the arsphenamines. All this was recognized and known by the workers who evolved the arsphenamines, and even then it was thought that the oxide was involved in or part of the scheme whereby the arsphenamines functioned as therapeutic agents. The possibility of using the oxide as the therapeutic agent was not overlooked, but for reasons of toxicity the matter was dropped. In using the oxide and its dichloride we are, therefore, not employing new chemical compounds but rather arsenicals that are essentially as old as the arsphenamines and what has been done is to find a way in which those old chemicals may be used safely and satisfactorily. To say this is not to detract one bit from the importance of the development that gave rise to this new way of using arsenicals for the treatment of syphilis, but to emphasize that the development was in its fundamentals a finding out of how to use something that already existed.

As the importance of the oxide type arsenicals became established, it was but natural that investigators would start to search the field of arsine oxides, testing those already known and synthesizing new ones for test. It is understandable that by means of a systematic study of this definite type of chemical compound a configuration might be found which would show higher effectiveness without any increase in toxicity and possibly with decreased toxicity. Moreover, there was the possibility that by testing a number of structures and by correlating the activity and toxicity data with structure, relationships might be discovered which would permit the visualization of the improved therapeutic agent desired. That this would be difficult to attain was apparent at the outset, because in the oxide structure corresponding to the arsphenamines satisfactory therapeutic effectiveness and comparatively low toxicity had been obtained, and the preparation

under conditions of clinical use did not cause frequent or serious reactions. In other words, the oxide preparation and its dichlor analogue already in clinical use are good therapeutic agents, and when that is the case, the discovery of something better is difficult as compared to a situation in which the drugs in use are comparatively ineffective. Thus, the chances of successful drug improvement are less. The volume of work done along these lines has been great, and much in the way of information has been accumulated; but the goal of an improved antisyphilitic oxide is still to be realized, and doubts as to whether such will be found become stronger. Success in this endeavor would have a profound effect on syphilis therapy. For example, it might make still more satisfactory the short, intensive methods of treating syphilis now coming into use. The value of such a result may justify the effort, but in any event the uncertainty of any new antisyphilitic arsenical or any changes in the method of treatment as a result of this work is a thing to keep in mind.

In the field of arsphenamines proper, the situation has not changed from what it has been for years in so far as the drugs in use are concerned. The field is still largely limited to the three products, arsphenamine, neoarsphenamine and sulfarsphenamine. In addition to the oxide type arsenical and the arseno or arsphenamine type, there is one more form of antisyphilitic arsenic—the arsonic acid. In this country tryparsamide (IV) continues to be the compound of importance in the arsonic acid field. Its structural formula, when compared with those given above, indicates how fundamentally different the arsonic acid type is from the others. Further, its usefulness is limited to the field of neurosyphilis as it is ineffective in other forms of the disease.



IV

From the standpoint of the arsenical drugs provided to the medical profession as antisyphilitics, there have been new constructive developments even though, excepting for the introduction of the oxide and its dichlor derivative into clinical use, there has been nothing new of value in the way of compounds. The arsphenamines as placed on the market have from the outset been subject to testing and approval by a government laboratory, now the National Institute of Health. The scope of this activity has been expanded so as to include the other types of arsenicals—the arsonic acid type and the oxide (or dichlorarsine) type. This has the advantage of assuring the user that no antisyphilitic arsenical preparation unapproved by this test laboratory can reach his hands. When the nature, size and importance of the syphilis problem are considered, when it is realized that the drugs are hazardous substances, and when one takes into account that the scale of treatment varies from the individual practitioner with an occasional case to large municipal clinics treating many cases, the importance of excluding unapproved material is obvious. The patient, the clinician and the manufacturer all are protected.

The scope of the control is not all that has expanded. The nature of the control is today different from what it was originally. Thus, certain of the test conditions are more severe, additional tests have been introduced and the practice of assigning to each lot an outdating has been adopted. This present arrangement is not a thing of the last year or two but is still a progressive development of recent times in the field of antisyphilitic arsenicals. The effect is to have for each type of arsenical a life period throughout which it may be expected to remain in good condition as a therapeutic material, assuming no mistreatment in the form of faulty storage for example, and to assure that nothing goes into the market which is not of such a character as to remain good for the intended period. The working out of these arrangements has been done in an understanding manner with full cognizance of the commercial and manufacturing problems involved. Full coöperation between scientific organizations, manufacturers

and Federal agencies has contributed in the public interest.

The antisyphilitic drugs as chemical substances constitute only one phase of the syphilis problem. Another phase deals with the manner in which the drugs are employed. The treatment of syphilis has always been and still is to all intents and purposes a long-drawn-out affair. When medication continues for a year and two years, the difficulties are obvious both from the standpoint of the individual and the matter as a national or health problem. The shortening of such a protracted period of medication to a single continuous treatment lasting for a period of days, or carried out as a short series of such intensive treatments, would constitute a development of tremendous importance which in its sphere is just as big a contribution as discovery of a new drug would be in its segment of the problem. That this may become reasonably commonplace, at least where proper facilities are available and conditions permit, is not too unlikely since it is already a reality in the sense that the method is being extensively investigated.

Much has recently been written of pioneer work in this field. First to be tried was the continuous drip method for a number of hours on five successive days, using neoarsphenamine in the beginning and then the less toxic oxide type of drug. The results were very encouraging except that frequent, sometimes severe, clinical reactions were encountered. As a result other abbreviated intensive treatment methods are being investigated. These include the administration of a number of small doses daily on successive or alternate days using only the oxide form of drug. More recently alternate therapy with the arsenical and fever treatments has been tried, also simultaneous use of drug and fever therapy. All of these methods have been reported as equally effective when compared to the longer established forms of treatment in the early stages of syphilis. However, none of them have yet been used on a sufficient number of cases, nor have these been observed for a long enough time to establish any one of these methods as superior to the others or equal in scope and result to the longer established methods. However, the work is still in progress. The tremendous value of a short and effective method for treating syphilis is obvious both from the public health standpoint and from the viewpoint of the individual patient. In addition to the important reduction in the treatment time is the other factor of reducing, if possible, the danger to the patient involved in treatment.

The patient is an important factor in the syphilis problem. The condition to be treated is one in which the infecting organisms are spread widely throughout the host, some in places where they may be reached with fair ease and others in places not easily reached. The drug used is a potent material which acts on the parasite because of toxicity which it has for that organism. However, this toxicity for the parasite activity expressed in another way—results in toxicity to a degree to the host. A high differential between the toxicities for the parasite and the host is the goal of all chemotherapy, but a specificity so high that the host toxicity is essentially nil is a condition which has not yet been achieved in this problem and it is therefore necessary to attempt, in the best possible way, to keep the effects of this host toxicity at a minimum. A certain amount can be done in this direction by keeping the drug as free as possible of toxic materials or properties. There is a limit to what can be done in this direction because once the drug has been brought down to a certain level of host toxicity one cannot go further. From that point on further progress can be made only by conditioning the patient so as to develop in him, as distinguished from the parasites in his body, a maximum resistance toward the host toxicity of the drug. To this end adequacy of vitamin intake is important. In particular natural vitamin C along with the things such as the P factor which accompany it may be used advisedly together with the various factors of the B complex.

Numerous attempts have been made to discover means by which the arsphenamines could be detoxified at the time of injection by using special solvents such as sodium

thiosulfate solution, sodium dehydrocholate, etc., but none of these devices has proved to be successful On the other hand, some of the studies which have been made with vitamin prevention of arsphenamine reactions have been strikingly successful, while others have not. The difficulty appears to lie in the fact that no attempt has been made to classify arsenical intolerances into those types which respond to preventive therapy with vitamins or drug therapy, and those which do not. When a sufficient number of cases has been studied and classified, the role of vitamin C, etc., in the prevention of arsenical reactions will be much clearer, and a more definite program for the conditioning of patients requiring arsenical therapy will be possible with a fuller understanding of what can be accomplished in this direction. This subject appears to present a field for fruitful and needed investigation.

The treatment of syphilis in the usual way, as distinguished from the short intensive method, still requires that a nonarsenical antisyphilitic be used as an adjunct. Ever since bismuth came into use for this purpose some twenty years ago, it has uninterruptedly been the preferred adjunct, and there are no indications that this will not continue to be the case. It is usually administered intramuscularly. For a while a few years ago it seemed as though a new kind of bismuth preparation might come into use modifying this adjunctional therapy in a fundamental manner. This was a material obtained by reacting sodium bismuthate and triisopropanolamine in propylene glycol. The reaction between these substances is a vigorous one and quite definitely results in new substances. However, what the nature or mechanism of the chemical reaction was, what the nature or identity of the resulting compound (or compounds) was, or even to what class of compounds the end products belonged was not established. Those were obviously matters of considerable complexity, and their solution would in the final analysis have little bearing on the question of whether the preparation did or did not have merit as an antisyphilitic. One form of this preparation was a heavy paste of a kind which could be put up in capsules for oral administration. Another form consisted of a solution for intramuscular injection. However, this latter did not constitute a new addition to the group of antisyphilitic bismuth preparations. It differed only in detail and not in type from what had already been in use for many years. The capsules for oral use did represent something new, and it was expected that by this means the management of syphilis would be facilitated measurably. For one thing the need for injections during the period of adjunctional therapy would be eliminated which, at least to the patient, would be a happy result. Bismuth in adequate amounts was absorbed when this preparation was administered orally, and it did appear that something new had been added to the list of bismuth products to be used along with the arsenicals. Nevertheless, the necessity for daily administration with an appreciable number of capsules per day and the tendency for the material to produce nausea constituted obstacles which prevented the preparation from reaching a point of extensive use. Accordingly, bismuth medication is essentially unchanged with the same assortment of preparations for administration by injection. However, it is interesting to note that whereas the arsenicals are all of one type in that they are water-soluble compounds, the group of bismuth products is varied in character and includes materials administered in water solution, glycol (nonwater solution), oil solution, oil suspension, water suspension and colloidal solution. The great variation in the form of the bismuth compounds used is due to the fact that intramuscular rather than intravenous injection is used thus permitting use of insoluble forms of bismuth compounds as well as the soluble forms.

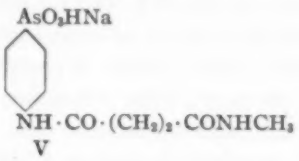
Neurosyphilis presents many problems of its own distinct from infections that have not reached that stage. The medication in such cases has not changed in character during recent years; the arsenical drug tryparsamide is used more or less routinely, and fever therapy is employed to advantage.

This latter is not new, but there have been developments in detail.

In addition to the use of tryparsamide in neurosyphilis, some success has been obtained by means of fever therapy. Elevation of body temperature is then accomplished either by intentional malaria infection,[3] the use of typhoid vaccine or by external application of ultra high-frequency currents.

The mechanism of fever therapy is not definitely established but the action is, in all likelihood, either a direct effect of the elevated temperature causing a change in the character and drug resistance of the infecting organism, a change in the host which causes an increased resistance to the organism, or a combination of several such factors. The advantage of fever therapy lies mainly in the fact that the effect occurs throughout the body and this is particularly important in neurosyphilis because the ability of the drugs used in this form of the disease to penetrate the central nervous system and their effectiveness is limited. Thus, the infecting organisms are difficult to reach, but in some cases at least fever therapy appears to offset this.

However, neither fever therapy nor tryparsamide alone is always effective in neurosyphilis. Some cases have received as many as 300 or more injections of the drug without cure or even satisfactory improvement. These cases are usually subjected, wherever possible, to fever therapy alone or alternately with courses or tryparsamide therapy if fever therapy is not successful. The results, even in these cases, are not altogether satisfactory, and many are resistant to the various limited means at present at hand. Further, tryparsamide has a specific toxicity for the optic nerve and, in some cases, cannot be used at all because of threatened destruction of the patient's vision. Recently a new drug called "neocryl" (V) has been introduced in England. The preliminary work has been promising but is not yet extensive enough to permit a thorough appraisal of its worth. Some claims have been made that it is less toxic to the optic nerve than tryparsamide and possibly more effective in neurosyphilis. If these are substantiated by extended use, definite progress will have been made in the handling of this most therapy-resistant and persistent form of syphilis. The formula of neocryl is shown (V).



V

The relation between the structures of neocryl and tryparsamide is apparent. Both are amino-substituted derivatives of *p*-arsanilic acid, both contain the —CONH— group, tryparsamide being an acetamido derivative and neocryl a succinylamido derivative. It is not surprising, therefore, that they have a similar effectiveness and field of application. Of the large number of arsonic acids synthesized only these and acetarsone, sometimes used orally in congenital syphilis, have attained any lasting usefulness as antisyphilitic agents.

From a review of the status of and developments in the syphilis problem the over-all impression obtained is that while progress has been made, it is progress in detail and not of the fundamental kind involving new kinds or methods of medication. This is not to say that the progress made has not been worth while. It has, and the management of syphilis today is better than it was in the past. However, there has been nothing revolutionary. This may be due to the fact that investigations have dealt with clinical procedures on one hand and with details of the chemistry of the antisyphilitic agents on the other. Little has been done on the problems underlying the whole subject. How do the arsenicals and other antisyphilitic drugs behave after injection; what happens after they are put into the blood stream; how do they act on the spirochete and kill it; how do they reach the spirochete in remote places and in what form are they by that time; etc.? It seems as though the problem of syphilis medication has reached the point at which these things must be understood if revolu-

[3] Any residual malarial infection is treated by the usual means—quinine, etc.

tionary changes and improvements are going to be made, assuming of course that anything different from and superior to present practices can be developed. It did seem that efforts along these lines were being started some years ago, but what obviously would have to be a very long-time project with no certainty of providing any improved scheme of medication, apparently lapsed after certain initial things had been accomplished. These efforts by several groups of investigators included studies on the relation of the reticulo-endothelial system to the effect of arsenical drugs on *Treponema pallidum*, the effect of glutathione on the activity of the arsphenamines, the formation of "arsenoxide" from arsphenamines, the anticoagulant effect of arsenicals on the blood, and a number of other widely dispersed phenomena. None of these investigations in themselves, or taken as a whole, present even a basis for an explanation of the mode of action of arsenical drugs in syphilis. None approach the problem from the viewpoint that three simultaneous considerations exist: namely, the action of the drug on the host, the action of the infecting organism on the host, and the action of the drug on the organism. The problem is tremendous, and it is small wonder that little progress has so far been made. But, until this work is done, achievement of the ultimate in syphilo-therapy can be attained only by good fortune rather than by thorough understanding.

One further discovery which eventually may lead to progress in this field is a recent development in the study of trypanosomiases such as kala-azar, leishmaniasis, etc., in England. Yorke and his collaborators have found that certain nonmetallic diamidines appear to be far more effective than antimony or arsenic compounds in dealing with these infections, while they are ineffective in dealing with syphilis or other diseases caused by spirochetes. It will be recalled that the effectiveness of atoxyl in trypanosome-infected rats was one of the underlying facts which led to the discovery of the arsphenamines. Work with the diamidines may lead to a new series of developments in the field of syphilo-therapy.

Recent Advances in Anesthesia*

John C. Krantz, Jr.†

INTRODUCTION

"Nothing in the whole realm of human effort has ever contributed so much to human comfort as the discovery of modern anesthesia."

Pain and discomfort are the arch enemies of man. To escape them and effectually combat them, he has ransacked the entire earth to find drugs to bring him a surcease of pain. During the middle of the 16th century Ambroise Paré operated without anesthesia, except for the administration of French wines, which would produce an alcoholic stupor. Only 120 years have passed since Ephraim McDowell removed an ovarian cyst from Mrs. Jane Crawford in Danville, Ky., without any anesthetic agent. She was then forty-seven years old and lived to see her seventy-eighth birthday. It is difficult for man today to appreciate the excruciating pain suffered by surgical patients in the preanesthetic days and, further, no one can with certainty estimate the impediment to surgical progress that the absence of anesthesia produced.

* This is the third of a series of review articles. It was scheduled for publication in December, but the December issue of THIS JOURNAL will be devoted to the Proceedings of the Ninety-first Annual Meeting of the A. PH. A.

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NITROUS OXIDE

Joseph Priestley, the discoverer of oxygen, prepared the first generally accepted anesthetic. Priestley was a Unitarian minister in Birmingham, England. In the congregation of this brilliant scientist-clergyman were three illustrious men: James Watt, who discovered the power of steam and holds the admiration of men in all walks of life; Erasmus Darwin, brilliant scientist and skilled clinician whose grandson, Charles, established a new order in biology; and William Withering, "Flower of English Physicians," discoverer of the use of the purple foxglove in edema of cardiac origin. In the year 1773, Joseph Priestley made nitrous oxide. To him it was a new chemical compound, a gas whose physical properties should be investigated. Priestley was unconcerned with its biological effects and died in Northumberland County, Pennsylvania, not knowing that nitrous oxide would confer a blessing of inestimable magnitude upon man.

A quarter of a century passed. Sir Humphry Davy, brilliant English chemist and physicist, made "Priestley's gas." It was then designated in chemical reports as "Dephlogisticated Nitrous Gas." Davy inhaled nitrous oxide and observed a period of great exhilaration with an increase in pulse rate. In a letter to one of his friends he wrote: "I danced around my laboratory like a madman." But further than this Davy observed that continued inhalation of the gas would produce insensibility to pain. In fact, Davy anesthetized certain of his friends to unconsciousness with nitrous oxide. On July 3, 1798, Mr. Wedgewood called on Davy and he used nitrous oxide on him. He recorded in great detail his experiences, which read, in most respects, like a patient's account of losing consciousness under nitrous oxide. Davy suggested the use of nitrous oxide in medicine, but nothing was done about it.

The scene shifted to America. In Hartford, Conn., on December 10, 1844, G. Q. Colton was delivering a lecture on popular science. Among the experiments performed by Colton was the apparent hypnotism of certain members of the audience, presumably by means of his gesticulations. Meanwhile one of Colton's associates engulfed the individual in nitrous oxide. This made effective the hypnotic art of Colton. That same afternoon, a dentist whose name was Horace Wells was in the audience. He saw one of the people swoon, fall and hit his head violently against a chair, without apparent sensation of pain. Through his scintillating intellect flashed the era of painless dentistry. The next day Wells persuaded one of his dental colleagues, Dr. Riggs, to extract one of his teeth, while under the influence of "laughing gas." Wells did not whimper. The first step in man's redemption from pain had been taken. Wells did not succeed in establishing the widespread use of his new anesthetic agent. In Boston, where he endeavored to employ it, the gas bag failed most inopportunely, and Wells was hissed out of the room as a mountebank and charlatan. When death came prematurely to Wells, he did not realize what a tremendous and far-reaching influence his observations would have upon the comfort and even the destiny of the race.

This present decade, therefore, marks one hundred years of use of nitrous oxide as a general anesthetic. During this period its popularity has waxed and waned, and during the last two decades the gas has definitely established itself for the smooth induction of ether-oxygen anesthesia.

Nitrous oxide is a colorless, odorless gas which is alleged to possess a sweet taste. The gas supports combustion only after the disintegration of the molecule into oxygen and nitrogen. Seeds cannot germinate or plants grow in an atmosphere of nitrous oxide. Nitrous oxide is very soluble in water, from two to three volumes of nitrous oxide dissolve in one volume of water. It is, however, like other general anesthetics, more soluble in oil than it is in water. Blood will dissolve a large volume of the gas. The gas does not combine with the homoglobin. Owing to its greater solubility in oil than in water, the gas as it is carried by the blood to the central nervous system partitions itself out of the blood into the lipids of the central nervous system producing narcosis.

Induction is prompt, narcosis occurring within one to two minutes. Anesthetists have set forth the following effects from the inhalation of various concentrations of nitrous oxide:

N_2O, Per Cent	O_2, Per Cent	Effect
80	20	Subconscious analgesia
84	16	Complete analgesia
89	11	Light anesthesia
94	6	Complete anesthesia, dangerous hypoxia with incomplete relaxation

Like many other volatile anesthetics, nitrous oxide enters the body and leaves it unchanged. In other words, it is refractory to catabolism by biological processes. Its anesthetic action according to modern concepts is apparently due to the production of a reversible oxygen-want in the central nervous system. By means of drugs, hypoxia of the central nervous system can be produced by at least three mechanisms.

1. Formation of carbonyl hemoglobin (carbon monoxide poisoning).
2. Inactivation of the cytochrome oxidase in the cells (cyanide poisoning).
3. Inactivation of the cytochrome reductase in the cells (narcosis-nitrous oxide, ether, chloroform, etc.)

Substances used clinically as anesthetics affect the cells of the central nervous system according to the third concept but, in addition, they exhibit the property of affecting, first, the cells of the cerebral cortex; second, those of the spinal centers; and, last, the cells of the vital medullary centers.

Inexplosibility, safety and availability are factors which give nitrous oxide a place of pre-eminence among the volatile general anesthetics.

ETHYL ETHER

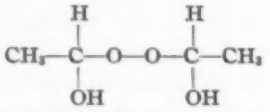
Crawford W. Long of Georgia used ether as a general anesthetic in 1842. He was familiar with some of the pharmacologic effects of ether and in Jefferson Co. there were many "ether frolics" which resembled modern parties of inebriates. He used ether also to deaden pain in the reduction of fractures and on Mr. James W. Venable to permit the surgical removal of a growth on the back of his neck. Unfortunate it is indeed that Long did not publicize his observations for apparently the first paper published by Long on ether appeared in 1849, five years after Wells' work with nitrous oxide, and three years after Morton's demonstration of the use of ether in Boston.

In Boston, a chemist named Jackson suggested the use of ether to a dentist named W. T. G. Morton, who was a pupil of Horace Wells. Morton persuaded Dr. J. C. Warren, grandson of Dr. J. M. Warren, associated with General Putnam at the Battle of Bunker Hill, to permit him to use ether on one of his patients. On October 16, 1846, in Massachusetts General Hospital, Morton began the administering of ether to Dr. Warren's patient. From that operating room reverberated that memorable statement which has echoed down through the decades, "Dr. Warren, your patient is now ready." Dr. Warren commented that this was no humbug. Mr. Abbott, the patient, was fast asleep. Ether had found its place.

Shortly after this Oliver Wendell Holmes, in a letter to Morton, conveyed the fact that he had assigned a generic name to ether and all such agents. He commented on the importance of a proper selection of a name, for Holmes held that it would be on the lips of every person of all races who in time to come would dwell on this planet. He coined the word "anesthesia" from the Greek ἀισθησις perception and the ἀν negative, namely, without perception. Ethyl ether is the most generally used of all volatile anesthetics. Its mode of action is like that of nitrous oxide. It is not decomposed in the body, but its presence in the cells of the central nervous system produces insensibility to pain and a hiatus in consciousness. Ether requires 6 to 8% concentration in the inspired air to produce anesthesia. During surgical anesthesia the concentration in the blood is approximately 150 mg. per cent. The blood pressure remains essentially normal, respiration full and regular during ether anesthesia. The relaxation of abdominal musculature is complete with ether. The administration of the anesthetic agent in the circuit with oxygen instead of air seems to

reduce the incidence of postanesthetic nausea and vomiting with ether. Postoperative abdominal distress and too great a degree of volatility appear to be the principal drawbacks to ethyl ether as an anesthetic.

Ether, when exposed to light and air, has a tendency to develop peroxides. These are explosive and also serve as pulmonary irritants, when ether containing them is employed as an anesthetic. The structure of ether peroxide, according to Wieland, is:



Dihydroxydiethyl peroxide

The Pharmacopœia requires that ether used for anesthetic purposes must be peroxide-free. The test is carried out as follows: "Shake 10 cc. of ether occasionally during 1 hr. with 1 cc. of a freshly prepared aqueous solution of potassium iodide (1 in 10) in a 25-cc. glass-stoppered cylinder of colorless glass, protected from light: when viewed transversely against a white background, no color is seen in either liquid."

CHLOROFORM

When the news of this important discovery of ether bridged the Atlantic, James Simpson of Edinburgh began an assiduous search for substances as good as, or perhaps better than, the American ethyl ether. It seems strange indeed that scientists in England did not precede those in America in the use of ether because Michael Faraday, distinguished pupil of Sir Humphry Davy, was one of the first chemists to produce ether. Simpson's experiments were fraught with many failures. Ether was better as a general anesthetic than most every substance that he and his associates tried. One day, as he was fumbling through the papers on his desk, he found a vial of a colorless liquid which had been sent to him by the German apothecary, Justus von Liebig. And Simpson tried it—the liquid was chloroform. Several times did he anesthetize himself and his associates, Keith and Duncan, to unconsciousness with at least apparent impunity. It is recorded that immediately Simpson recommended the use of chloroform to alleviate the pain of childbirth. To this the clergy of England objected. They contended that this pain was a penalty pronounced upon Eve for her transgression in the garden of Eden and in consequence all subsequent generations of women should endure it with patience and complacence. Simpson was a careful investigator, but also was astute at repartee. To this criticism he very aptly replied, "The Lord caused Adam to fall into a deep sleep before appropriating his rib; out of which he created Eve." God administered the first anesthetic. Queen Victoria, that pioneer of English customs, broke the spell of superstition by permitting Simpson's chloroform to be used in her seventh confinement.

Chloroform is about five time more potent than ether as an anesthetic. It is also much more toxic. The decline in the use of chloroform in general anesthesia is due to its striking toxic action upon the heart and liver. Ether does not exhibit this. Most deaths under chloroform anesthesia occur in the induction stages. The mechanism of the acute intoxication probably takes place in the following manner. Chloroform stimulates the vagus centrally and slows the heart. Through the excitation of induction endogenous epinephrine stimulates the cardiac accelerator, thus the simultaneous effect of stimulation and depression of the heart rate produces fibrillation of the auricles and ventricles. In addition, chloroform is carried from the alveolar air to the left chambers of the heart in concentrations which are cardiotoxic. Before this has been diluted by the general circulation, cardiac stoppage is produced. The approximate mortality under chloroform anesthesia is of the order of magnitude of 1 in 2500; with ether, 1 in 10,000. It is of interest to note that the fluorine analogue of chloroform, namely fluoroform, is neither anesthetic nor toxic.

ETHYLENE

After the discovery of chloroform there followed several barren decades in the field of general anesthesia and at the turn of the

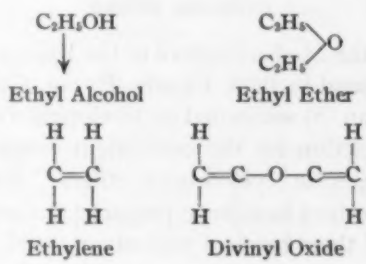
century the armamentarium of the anesthetist contained only nitrous oxide, ether and chloroform augmented in a small measure by ethyl chloride. Through the first two decades of the present century no substantial gains were made. However, in 1922, ethylene was introduced by Luckhardt of the University of Chicago. It had been observed that traces of ethylene caused the bleaching of pigmented flowers and this observation had come to Luckhardt's attention. He was curious about it. He wondered what effect ethylene would have upon animal protoplasm. Systematically he tested the gas on lower animals and observed its anesthetic effects. Ascending in the scale of development, he observed that the anesthetic properties held for monkeys and finally he permitted himself to be anesthetized many times to unconsciousness and ethylene took its place among the general anesthetics. Perhaps this discovery illustrates the characteristics of a scientist, "one who has the simplicity to wonder, the ability to question, the power to generalize and the capacity to apply." High concentrations of ethylene are required to produce anesthesia (85 to 90%) and, to avoid hypoxia, the gas must be administered with oxygen. The gas mixture is extraordinarily explosive, and many tragic accidents have occurred owing to the explosion of the gas through ignition by static electric sparks. Undoubtedly this has militated against the wisespread use of the gas in many places.

AVERTIN

In 1927, Willstätter prepared a general anesthetic, tribromethanol, marketed and employed as avertin, dissolved in amylene hydrate. The principle involved in this discovery is based upon the theory of narcosis announced by Meyer and Overton in 1900. Essentially this theory holds that the greater the oil/water solubility is, the more potent is its activity on the central nervous system. Alcohol has anesthetic properties. The alcohols of the aliphatic series of hydrocarbons of higher molecular weight such as amyl and octyl alcohols are less water soluble and more oil soluble, and their potencies as anesthetic agents are greater than that of ethyl alcohol. In avertin, three of the hydrogen atoms of the ethyl alcohol molecule having a combined atomic weight of 3 have been replaced by three bromine atoms, the sum of whose atomic weight is approximately 240. This increase in molecular weight increases the oil/water coefficient and simultaneously enhances the anesthetic potency of the compound. Avertin is administered rectally. Its anesthetic index or safety margin is narrow, *i. e.*, the anesthetic and fatal doses do not vary by a great degree of magnitude. Therefore, most anesthetists prefer to use the drug in amounts equal to three-quarters of its anesthetic dose as a basal anesthetic and to complete the relaxation with nitrous oxide or ether. The drug is contraindicated in patients suffering with hepatic or kidney diseases. It is unfortunate that all of our data on the efficacy and safety of tribromethanol are befogged by the fact that it is employed dissolved in another anesthetic agent, namely, amylene hydrate. Furthermore, avertin is a fixed anesthetic, and threatened collapse under agents of this kind is much more difficult to combat than it is under volatile anesthetics. Under the latter, removal of the mask initiates the immediate course of removal of the agent from the circulating blood. Obviously, when a fixed anesthetic agent is used, this safety factor in unavailable.

DIVINYL OXIDE

It occurred to Chauncey Leake (1) of the University of California in 1930 that it would be of great interest to prepare a hybrid molecule between ethyl ether and ethylene, *i. e.*, a molecule which contained



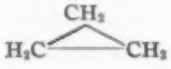
the essential features of the molecules of each of these anesthetics. Following this

suggestion Major and Ruigh (2) prepared divinyl oxide, "Vinethene." The relation of these compounds to ethyl alcohol can be seen from the formulas.

Divinyl oxide is more powerful than ether. It is a liquid of very low boiling point. With it, anesthesia is rapidly induced, but owing to hepatic injury which may occur upon prolonged inhalation of this anesthetic agent, its use is confined to operations of short duration. One must not pass over the production of this new agent without paying due tribute to the fertility of the mind that conceived it. In its conception a molecule was designed, synthesized and anticipated properties were later discovered to be inherent in it.

CYCLOPROPANE

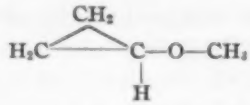
In 1930 Lucas and Henderson (3) of the University of Toronto announced the anesthetic properties of the hydrocarbon, cyclopropane. The structure of cyclopropane is seen in the following formula:



The gas is more potent than ethylene and hence permits the admixture during anesthesia of a larger percentage of oxygen. Relaxation of abdominal musculature is good during cyclopropane anesthesia. During the decade of its use the gas is now established as an important and dependable agent. Recognition of it in the United States Pharmacopœia XII bespeaks its growing field of usefulness. A brochure by Robbins (4) of Vanderbilt University on cyclopropane reviews the entire field.

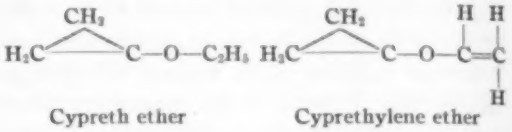
CYPROME ETHER

At the Medical School of the University of Maryland in 1939, Krantz, Evans, Carr and Forman (5) succeeded in developing a chemical reaction for the convenient preparation of aliphatic cyclopropyl ethers. Four of these ethers have been prepared already, and one of them has had preliminary trial. This new anesthetic agent is cyclopropyl methyl ether known as cyprome ether; its structure can be seen by the following formula:



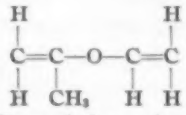
The pharmacologic studies conducted in the University of Maryland show cyprome ether to be more potent than ethyl ether and possibly safer. Its boiling point is 10° C. higher than ethyl ether which should be a distinct advantage for anesthesia in the tropics. Black, Shannon and Krantz (6) in 1940 reported their first 25 human cases of anesthesia with cyprome ether in "Anesthesiology." The compound appears to be promising.

Other new anesthetics which have been produced by these investigators and are under study at the present time are:



Cypreth ether Cyprethylene ether

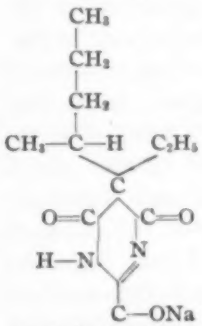
and



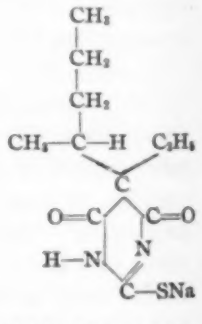
Propethylene ether

PENTOTHAL SODIUM

Many years ago Fränkel prepared certain thiobarbituric acid compounds. Based upon insufficient evidence upon a very limited number of animals these compounds were discarded as not being worthy of therapeutic merit. Within the past five years these compounds have been reinvestigated and found to be very prompt-acting barbiturates, yet the action is of very short duration. The one most extensively used in this country is pentothal sodium. Structurally



Pentobarbital sodium (Nembutal sodium)



Pentothiobarbital sodium (Pentothal sodium)

this compound is pentobarbital in which the oxygen atom of the urea group has been replaced by a sulfur atom.

The widest use of this compound very recently has been in producing anesthesias by intravenous injection for surgical procedures of short duration. For this purpose 2 to 3 cc. of a 5% solution is injected in about 15 seconds. The injection is then discontinued to permit the complete effect to become manifest, which requires about 35 seconds. If relaxation has not occurred, an additional 2 to 3 cc. may be slowly injected.

The importance of pentothal sodium as a combat anesthetic cannot be overestimated. The mortality statistics with pentothal sodium are favorable and this agent appears to have warranted a permanent and enviable position among the anesthetic agents.

SPINAL ANESTHESIA

The use of local anesthetic agents in the spinal fluid to produce anesthesia dates back to the turn of the century. In the early days of this form of anesthesia, the deaths were so numerous that the popularity diminished. In more recent years, with more skillful technique and more numerous new synthetic local anesthetic agents to select from, this type of anesthesia has received a new impetus and is at present enjoying much popularity.

When a local anesthetic is injected into the spinal fluid impulses over all types of nerve fibers are blocked—sensory, motor, somatic and autonomic. The sensory block occurs in six to eight minutes which is followed by motor paralysis. The duration of the insensibility to pain and the motor paralysis is a function of the character and the concentration of the local anesthetic agent. Procaine hydrochloride in safe concentrations produces an anesthesia of one-hour duration. Tetracaine hydrochloride (pontocaine) can be successfully used for a period of three hours.

There are many side reactions that may occur after the injection of an anesthetic agent intrathecally. There is a loss of motor tone which follows the paralysis of the vasoconstrictor fibers in the anterior nerve roots. The blood pressure consequently may fall to a very low level requiring the use of such drugs as ephedrine. Radiculitis, neuritis, meningitis and palsies are some of the undesirable anesthetic sequelae that have occurred with the use of spinal anesthetics.

OUTLOOK FOR THE FUTURE

Perhaps there is no field in medicine where achievements have been as great and so far reaching as have been the advances in anesthesia. The scope of this review purposefully excluded advances in the techniques of anesthesia such as the invention of the anesthetic rebreathing machines, the endotracheal tube, the use of moist soda lime and conducting rubber devices for the grounding of static sparks. All of these have made definite contributions to anesthetic success and safety.

Future researches are certainly to be directed toward the end of developing a better volatile anesthetic agent than ether. It appears indeed to be possible. Intravenous anesthesia needs to be made safer. Other agents need to be investigated further and better antidotes than are now available must be found. The future must investigate further and understand more clearly from the point of view of cellular physiology, what is meant by that profound hiatus in consciousness, so glibly referred to as surgical anesthesia.

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(3) Lucas, G. H. W., and Henderson, V. E., *Can. Med. Assoc. J.*, 21 (1929), 173.

(4) Robbins, B. H., "Cyclopropane Anesthesia," First edition. Williams and Wilkins Co., Baltimore, Md., 1940.

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Preparations of Phenylmercuric Benzoate and Their Bactericidal Value*

By B. Baront and H. G. DeKayt

Certain organic mercury compounds which have been discussed lately in the literature are included in a group designated by such terms as phenyl mercury, merphenyl, phenylmercuric, mercury phenyl and others. Available literature indicates that phenylmercuric nitrate was first discussed in 1931 (1) as a disinfectant but was first prepared in 1870 by Otto (2). Since 1931, many compounds of the phenylmercuric salts have been prepared such as the citrate, acetate, benzoate, borate, chloride, nitrate, sulfate, phosphate, oxalate, picrate, tartrate, carbonate, cinnamate, acrylate, lactate and atropate.

Phenylmercuricnitrate appears to have received the most attention. It has been recommended in a variety of dilutions for various purposes. The Hamilton Laboratories, Inc., Hamilton, Ohio (3), have obtained a copyright on the term "Merphenyl" for their mercury phenyl compounds.

The purpose of this investigation was to make preparations which have germicidal properties and which could be used by the Student Health Service of Purdue University. The phenylmercuric compounds offered a possible solution to our problem. The one with which we were concerned was phenylmercuric benzoate.

In making a survey of literature of phenylmercuric benzoate we found very few references as to its properties and its use. Armangue and Maestres (4), in 1934 discussed several of the phenylmercuric salts which they had synthesized. They also discussed the bacteriacidal action of these salts among which was the benzoate. From their report, the melting point is 97 to 99° C The solubility of phenylmercuric benzoate is 0.80 Gm. in 1000 cc. of water at 15° C., 48.90 Gm. in 1000 cc. 98% ethyl alcohol at 10° C., and 10.00 Gm. in 1000 cc. neutral or pure glycerin at 15° C.; very soluble in chloroform and ether. It is a tasteless, white amorphous powder with a soft velvety texture. The Bureau of Internal Revenue mentioned it as one of the compounds that could be substituted for potassium iodide in the modified S. D. A. formula No. 42 for suture tubing fluid (5).

Phenylmercuric benzoate was found to be soluble in other organic solvents such as acetone and diethylene glycol monoethyl ether(carbitol). We also found that sodium citrate increases the solubility of this compound in aqueous solutions.

Solutions were prepared of varying concentrations in aqueous, alcoholic and alcoholic combined with other organic solvents.

Ointments of phenylmercuric benzoate were prepared using a number of different ointment bases which included both the fatty and nonfatty types.

The inhibitory power of the different preparations was determined by using the standard agar-cup method with nutrient agar as the medium.

EXPERIMENTAL

Solutions of phenylmercuric benzoate were prepared in concentrations of 1:1000 to 1:24,000. These solutions were stored in clear glass bottles. It was found that those solutions containing organic solvents changed in color and were evidently so affected by light.

Ointments containing phenylmercuric benzoate 1:1000 and 1:6000 were prepared in bases such as aquaphor, white petrolatum with 2% cholesterol, Simple Ointment, U. S. P., and others.

Commercial antiseptics were obtained on the open market and will be found in the accompanying experiments and tables designated as A, B, C, D and E. A series of bactericidal tests were carried out to determine the inhibitory power of the phenylmercuric benzoate and other compounds on *Staphylococcus aureus*, 1041, and *Eberthella typhi*, P. D. The agar-cup method was used in testing the antiseptic properties.

The beef broth was prepared by using 5 Gm. of Armour's beef extract, 5 Gm. of sodium chloride

* Received June 1, 1943.

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‡ Professor of Pharmacy, Purdue University.

Fig. 1.

and 10 Gm. of Armour's peptone in 1 L. of distilled water. One and one-half per cent of granulated agar was then added and solution effected. The nutrient agar having been made was adjusted to a *p*H 6.8 with 2% sodium hydroxide and then filtered. Fifteen cubic centimeters of the nutrient agar was placed in test tubes, the tubes plugged with cotton and finally sterilized at 15 lb. pressure for 30 min.

Ten cubic centimeters of sterilized physiological salt solution was added to each of the previously prepared 24-hr. slant cultures of the organisms. These were shaken, then strained through cotton.

The agar which had been previously tubed and sterilized was melted and cooled to 45° C. The tubes were divided into two sets, 0.1 cc. of the culture of *S. aureus*, 1041, and 0.1 cc. of the culture of *E. typhi*, P. D., were added to each set, respectively. The inoculated agar was then poured into a sterilized Petri dish and allowed to harden, then a hole was bored in the agar with a sterile cork borer.

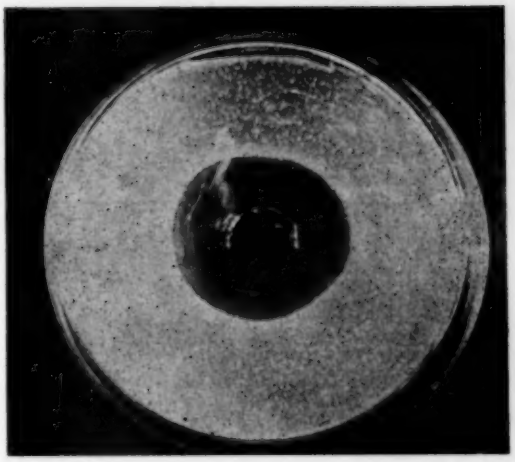

Fig. 3.

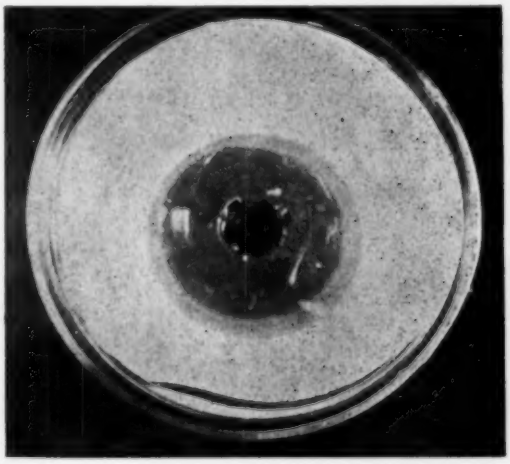

Fig. 2.

A drop of melted agar was then introduced by means of a pipette into the hole to form a thin membrane in order to complete the cup. A drop of the prepared solution was introduced into the cup by means of a standardized pipette. The plates were incubated for 72 hr. at a temperature of 37° C., with the results shown in Table I. One can see that the solvents used by themselves produced no inhibitory power on the organisms; therefore the zone which has been produced is due to the chemical which was used in the solution. Results of Table I have been duplicated a number of times.

It is to be noted that solutions of phenylmercuric benzoate in concentrations of 1:1000 to 1:24,000 offered greater zones of inhibition than that of phenol in concentrations of 1:100 and 1:10,000. The zones of inhibition given by alcohol-acetone combination were greater than those of other organic solvents and by the aqueous solutions. The alcohol-acetone

TABLE I

Formula	Concentration	Inhibition *S. aureus*	Inhibition *E. typhi*	Zone Inhibition, Cm. *S. aureus*	Zone Inhibition, Cm. *E. typhi*
2[a]	1:1000	*f*	*g*	2.00	1.20
3[a]	1:1000	*g*	*g*	1.00	1.00
4[a]	1:1500	*f*	*g*	1.20	1.00
5[a]	1:3000	*f*	*g*	1.50	0.50
7[b]	1:2000	*f*	*g*	1.00	1.00
11[a]	1:3000	*f*	*g*	1.50	1.10
12[c]	1:3000	*g*	*g*	1.50	1.50
14[d]	1:1000	*f*	*f*	1.70	1.70
15[d]	1:000	*g*	*g*	1.70	1.50
16[a]	1:6000	*g*	*g*	1.00	1.50
17[b]	1:24,000	*g*	*g*	1.00	1.30
18[a]	1:18,000	*f*	*f*	1.00	1.40
19[c]	1:12,000	*f*	*g*	1.20	1.70
20[d]	1:6000	*f*	*g*	2.00	1.60
21[d]	1:12,000	*f*	*f*	1.00	1.00
22[e]	1:1000	*f*	*f*	3.00	3.00
23[e]	1:500	*f*	*f*	2.75	3.00
24[e]	1:200	*f*	*f*	2.75	2.75
Sol. A	1:1000	*g*	*g*	1.70	1.50
Tr. A	1:500	*g*	*g*	2.00	1.50
Sol. B	1:1000	*g*	*g*	1.50	1.00
Tr. C	1:1000	*g*	*g*	3.00	3.00
Sol. C	1:1000	*g*	*g*	2.70	3.00
Tr. D	1:200	*g*	*g*	3.00	2.50
Sol. D	1:500	*g*	*g*	1.50	1.50
Tr. E	1:1000	*g*	*g*	2.00	2.40
Alcohol	95%	..	..	..	..
Phenol	1:100	..	..	..	..
Phenol	1:10,000	..	..	..	..
Acetone	....	..	..	..	..
Carbitol	....	..	..	..	..
Ether	....	..	..	..	..

[a] A C Ac.
[b] A C.
[c] A C E.
[d] Aqueous solution.
[e] A Ac.
f Zone of inhibition very clear.
g Zone of inhibition clear.

A, Alcohol.
C, Carbitol.
Ac, Acetone.
E, Ether.

TABLE II

Formula	Concentration	Base	Inhibition *S. aureus*	Inhibition *E. typhi*	Zone Inhibition, Cm. *S. aureus*	Zone Inhibition, Cm. *E. typhi*
63[a]	1:1000	Aquaphor	*f*	*f*	1.75	2.50
64[b]	1:1000	Aquaphor	*f*	*f*	3.00	1.50
61[c]	1:1000	Aquaphor	*f*	*f*	1.00	1.75
56[b]	1:1000	White petrolatum plus 2% cholesterol	*f*	*f*	1.25	2.00
55[d]	1:1000	White petrolatum plus 2% cholesterol	*f*	*f*	1.50	2.50
53[c]	1:1000	White petrolatum plus 2% cholesterol	*f*	*f*	1.50	1.50
45[c]	1:1000	Simple Ointment, U. S. P.	*f*	*f*	2.00	2.00
1[e]	1:6000	Cetyl alcohol	*f*	*f*	1.50	1.50
2[e]	1:1000	Aquaphor	*f*	*f*	2.50	2.50
3[e]	1:6000	Aquaphor	*f*	*f*	3.00	3.00
4[e]	1:1000	Simple Ointment, U. S. P.	*f*	*f*	2.00	2.00
5[e]	1:6000	Simple Ointment, U. S. P.	*f*	*f*	2.50	2.00
6[e]	1:1000	White petrolatum plus 2% cholesterol	*f*	*f*	2.00	1.75
7[e]	1:6000	White petrolatum plus 2% cholesterol	*f*	*f*	1.50	1.50

[a] Chloroform used as solvent for phenylmercuric benzoate.
[b] Acetone used as solvent for phenylmercuric benzoate.
[c] Alcohol used as solvent for phenylmercuric benzoate.
[d] Ether used as solvent for phenylmercuric benzoate.
[e] Water used as solvent for phenylmercuric benzoate.
f Zone of inhibition very clear.

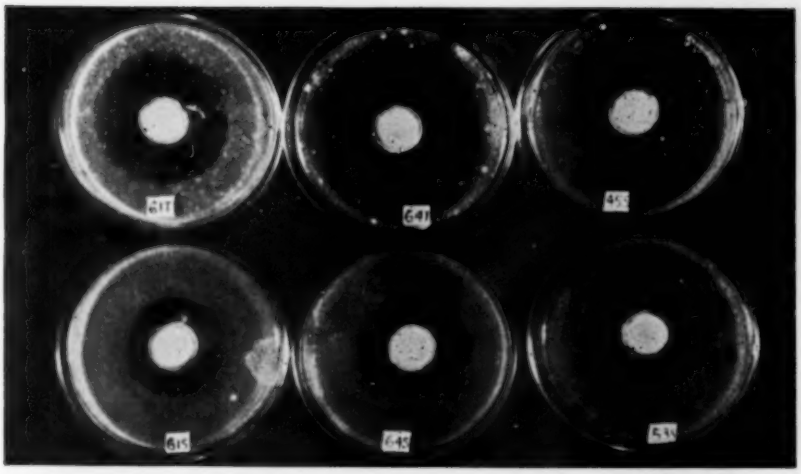

Fig. 4.

solutions are comparable to some of the commercial antiseptics as shown by the table. (Figs. 1, 2, 3.)

The antiseptic property of 1:1000 and 1:6000 phenylmercuric benzoate in the various ointment bases was then tested by preparing nutrient agar and plates with the same procedure as above except the hole was not bored in the agar. Microsublimation rings, 3 x 15 mm., were filled with the ointment to be tested and were then placed on the hardened medium. The plates were incubated at 37° C. for 72 hr., with the results shown in Table II.

From Table II it is to be noted that the ointments of phenylmercuric benzoate in concentrations of 1:1000 to 1:6000 in aquaphor and Simple Ointment, U. S. P., base offered greater zones of inhibition than those in other ointment bases. The zones, given by the ointments in which the phenylmercuric benzoate had been dissolved in water, were greater than when an organic solvent was used. (Figs. 4, 5, 6.)

SUMMARY AND CONCLUSION

1. Aqueous solutions of phenylmercuric benzoate 1:1000 to 1:6000 inhibit growth of *S. aureus*, 1041, and *E. typhi*, P. D.

2. The solutions of phenylmercuric benzoate in the alcohol-acetone combination produce greater zones of inhibition than other combinations of organic solvents.

3. The solution of phenylmercuric benzoate in the alcohol-acetone combination produce greater zones of inhibition than those of the aqueous solutions.

4. Ointments of phenylmercuric benzoate 1:1000 to 1:6000 inhibit the growth of *S. aureus*, 1041, and *E. typhi*, P. D.

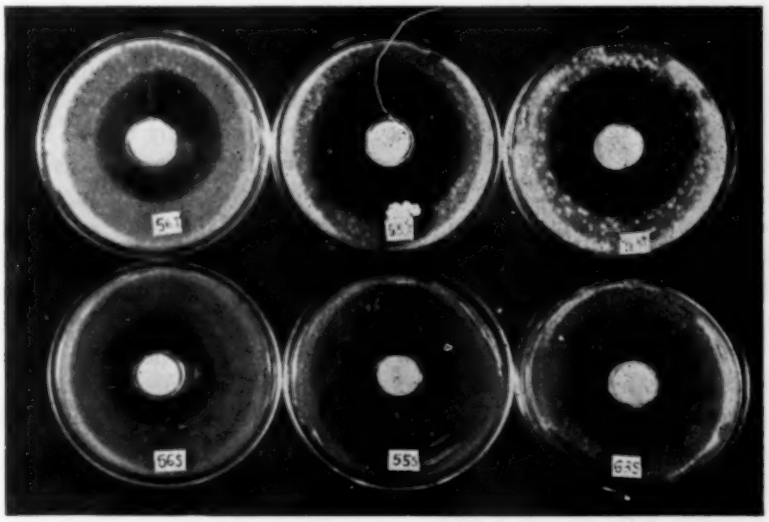

Fig. 5.

Fig. 6.

5. Ointments made with aquaphor and Simple Ointment, U. S. P., as bases produce greater zones of inhibition than other ointments made with other bases.

6. The ointments which produced greater zones in the above bases were those in which water was used as the solvent.

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A Comparison of Methods Used for Evaluating the Bactericidal Properties of Mercurial Compounds*

By Bernard Heinemann†

Reports of the bactericidal effectiveness of mercury preparations do not seem to be in harmony due mainly to the misinterpretation of reported results and the lack of a suitable *in vitro* test for their evaluation. The known mercurials possess high bacteriostatic properties and, as a result, tend to give falsely high phenol coefficient values. The need for an *in vitro* bactericidal test using suitable recovery media containing, if known, neutralizing substances for the preparation under test, in the evaluation of surface disinfectants, has been recognized (1).

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The high inhibitory property of mercurial compounds, preventing growth in the subcultures of the phenol coefficient test, was recognized by the U. S. Department of Agriculture in Circular 198. It was recommended that subcultures should contain very large amounts of medium or they should be retransferred by carrying at least 4 loopfuls from the first subculture to a second tube of broth as recommended by Shippen (2). Unduly high and misleading bactericidal values have been given in the case of mercurial disinfectants since in many cases neither of these methods has been followed.

There are many sulfur-containing compounds which can neutralize the bacterio-

static activity of mercurial preparations and permit bacteria which are inhibited but not killed by the mercurial to multiply further in a suitable culture medium. It should be noted that many sulfur-containing substances are present in the human body.

Fildes (3) suggested that mercurial compounds were effective as antibacterial agents due to their interference with the sulfur metabolism of the organisms. He found that equimolecular quantities of sulfur would neutralize the mercurial after it had acted on the bacteria and permit them to grow in culture. Hunt (4) subjected bacteria, which had been washed and centrifuged after exposure to a mercurial, to hydrogen sulfide and found that they would then grow in a culture medium. The U. S. P. XII (p. 611) recommends sodium thiosulfate in testing the sterility of sutures as a neutralizer for mercury when it is employed as a bacteriostatic agent in the tubing fluid used in the packaging. Marshall, Gunnison and Luxen (5) used a thioglycollate medium to determine the sterility of serums and biologicals preserved with mercurials. Daily and Blubaugh (6) suggested using a thioglycollate culture medium in evaluating the bactericidal properties of mercurial compounds to neutralize their bacteriostatic properties. Blubaugh and Reed (7) found sodium thioglycollate medium to be superior to ordinary infusion broth for testing biological preparations preserved with mercurials. Nungester, Hood and Warren (8) obtained a higher percentage of positive cultures when staphylococci treated with various mercurials were subcultured to thioglycollate broth than when transferred to plain broth. A concentration of 0.5 to 0.10% thioglycollate was necessary to produce this effect. Hoyt, Fisk and Burde (9) found the mercurials tested to have low bactericidal values when sodium thioglycollate broth was used as a subculture medium.

The experiment to be described was devised to compare the results obtained on the bactericidal activity of various mercurial preparations using (*a*) Shippen retransfer technique, (*b*) 100 cc. of plain broth for subcultures and (*c*) thioglycollate medium for subcultures.

EXPERIMENTAL

Twenty-four-hour broth cultures of *Staphylococcus aureus* No. 209 were used as test organisms. Various dilutions of the preparation were made in 5-cc. volumes in sterile distilled water. One-half cubic centimeter of test culture was pipetted into 5 cc. of each preparation at 20° C., mixed, and allowed to stand at this temperature. After 5-, 10- and 15-min. intervals, subcultures were made with a 4-mm. loop (of No. 23 B. and S. gauge wire) into (*a*) 10 cc. of nutrient broth, shaken, and then 4 loopfuls transferred to a second 10-cc. tube of nutrient broth, (*b*) 100 cc. of nutrient broth and (*c*) 10 cc. thioglycollate medium. All subcultures were incubated at 37° C. for at least 14 days and observed for growth.

The following mercurial preparations were used in the tests: (1) Sodium 4-nitro anhydro hydroxy mercury orthocresol, 1:500 aqueous; (2) Sodium ethyl mercuri thiosalicylate, 1:1000 aqueous; (3) Sodium ethyl mercuri thiosalicylate, 1:1000 tincture; (4) 2-Hydroxy mercuri furan, 1:3000 aqueous; (5) 2-Hydroxy mercuri furan, 1:400 tincture; (6) Secondary amyl tricresols and ortho hydroxy phenyl mercuric chloride, 1:1000, of each, tincture; (7) Sodium oxymercury ortho nitro phenolate, 1:1000 aqueous; (8) Ortho hydroxy phenyl mercuric chloride, 1:250 in 30% acetone; (9) Mercuric chloride, 1:500 aqueous.

Preparations 1 to 6, inclusive, were obtained commercially ready for use. Solutions of preparations 7 and 9 were made from the dry material, obtained commercially. Preparation 8 was synthesized in this laboratory and the solution prepared. It was found that the bactericidal effect of the alcohol, at 20° C., in the tincture solutions could be eliminated by dilution of the tincture, with sterile distilled water, to a 25% concentration of alcohol, by volume.

The culture media used were nutrient broth (Difco) and thioglycollate medium (Baltimore Biological Laboratory). The thioglycollate medium had the following composition:

INFUSION FROM 37.5 GM. OF MEAT

	Per Cent
Peptone, thio	1.0
Dextrose	1.0
Sodium chloride	0.5
Dipotassium phosphate	0.2
Sodium thioglycollate	0.1
Agar	0.05
Methylene blue	0.0002

RESULTS

The results are summarized in Table I and indicate the extent to which the preparations can be diluted with water and yet kill *S. aureus* No. 209 on 5-, 10- and 15-min. exposure at 20° C. The values given, multiplied by the original dilution of the preparation, indicates the highest bactericidal dilution.

Both sodium ethyl mercuri thiosalicylate, 1:1000, aqueous, and 2-hydroxy mercuri furan, 1:3000,

aqueous, were lacking in bactericidal properties, by the 3 methods tested, in their lowest dilutions as supplied for use. Sodium ethyl mercuri thiosalicylate, 1:1000 tincture, did not exhibit killing activity when diluted in half, which is necessary to eliminate the killing action, by dilution, of the alcohol present in the tincture.

Bactericidal dilutions could not be obtained for 2-hydroxy mercuri furan, 1:400 tincture, using thioglycollate medium for subculture, when the killing action of the alcohol was eliminated, by dilution. Using 100 cc. nutrient broth for subculture, a 1:800 dilution appeared to be lethal and, with the Shippen procedure, a 1:1000 dilution; both after 10 min. for subculture. With the Shippen method a three- and four-fold dilution of it appeared to be able to kill after 10- and 15-min. exposure, respectively.

Ortho hydroxy phenyl mercuric chloride, 1:250 in 30% acetone, could be diluted slightly and still kill the test organism when thioglycollate medium was used for subculture. With the other 2 subculture methods it apparently could be diluted to a much greater extent and still show killing action.

Mercuric chloride, 1:500 aqueous, did not show evidence of bactericidal properties when the thioglycollate medium was used for subculture. However, higher dilutions of it appeared to kill when tested according to the other 2 methods.

TABLE I.—COMPARATIVE BACTERICIDAL ACTIVITY OF MERCURIALS[a]

Description of the Preparation	100-Cc. Broth Culture, Min.			10-Cc. Thioglycollate Medium Subculture, Min.			Shippen Retransfer Method, Min.		
	5	10	15	5	10	15	5	10	15
1. Sodium 4-nitro anhydro hydroxy mercury orthocresol, 1:500 aqueous	3	5	6	[b]	[b]	[b]	[b]	[b]	[b]
2. Sodium ethyl mercuri thiosalicylate, 1:1000 aqueous	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]
3. Sodium ethyl mercuri thiosalicylate, 1:1000 tincture	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]	[c]
4. 2-Hydroxy mercuri furan, 1:3000 aqueous	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]
5. 2-Hydroxy mercuri furan, 1:400 tincture	[c]	2	2	[c]	[c]	[c]	[c]	2.5	2.5
6. Secondary amyl tricresols and ortho hydroxy phenyl mercuric chloride, 1:1000, of each, tincture	20	20	25	15	15	20	15	20	25
7. Sodium oxymercury ortho nitro phenolate, 1:1000 aqueous	[b]	[b]	[b]	[b]	[b]	[b]	[b]	3	4
8. Ortho hydroxy phenyl mercuric chloride, 1:250 in 30% acetone	8	12	20	1.2	1.6	1.6	3	12	12
9. Mercuric chloride, 1:500 aqueous	1	2	2.5	[b]	[b]	[b]	1.8	2.2	2.4

[a] Highest dilution of the preparation which kills *S. aureus* on different periods of exposure at 20° C.
[b] Growth occurred in subculture in lowest dilution of the preparation.
[c] Growth occurred in subculture in lowest dilution of the tincture preparation which eliminated the killing action of the alcohol.

Sodium 4-nitro anhydro mercuri ortho cresol, 1:500 aqueous, was incapable of killing *S. aureus* after 15-min. exposure when the thioglycollate medium was used for subculture. On the other hand, with the Shippen retransfer method, results indicate that a 1:1000 dilution was able to kill after 15 min. Using 100 cc. of broth for subcultures, even higher dilutions appeared to be capable of killing after 5-, 10- and 15-min. exposures.

Secondary amyl tricresols and ortho hydroxy phenyl mercuric chloride, 1:1000 of each (tincture), showed marked killing action even when diluted 15 or more times, by all 3 methods of testing. Sodium oxymercuri ortho nitro phenolate, 1:1000 aqueous, could not be shown to have bactericidal activity in up to 15-min. exposure, when tested, using 100 cc. of broth and 10 cc. thioglycollate medium

DISCUSSION

The results presented indicate that a neutralizing medium for subculture, such as the thioglycollate medium used, is more satisfactory in the evaluation of the bactericidal properties of mercurial preparations than either of the two "dilution" methods used. In several cases falsely high bactericidal dilution values could be reported using the "dilution" techniques. With sodium oxymercuri ortho nitro phenolate, 1:1000 aqueous, the absence of killing activity was noted with both the 100-cc. broth and the thioglycollate medium subcultures, but a higher bactericidal dilution appeared when the Shippen method was used.

It is interesting to note the low bactericidal activity *in vitro* against *S. aureus* of several of the proprietary preparations in the dilutions available

which enjoy widespread use as antiseptics and skin disinfectants.

The high bactericidal activity exhibited by secondary amyl tricresols and ortho hydroxy phenyl mercuric chloride, 1:1000 of each (tincture), was probably not due to the mercurial, but rather to the presence of secondary amyl tricresols.

The use of "dilution" subculture methods for determining *in vitro* bactericidal dilutions of mercurials is based on the assumption that merely placing the mercurial-bacterial mixture in a large enough amount of medium will eliminate the bacteriostatic action of the mercurial. The work of Fildes (3) suggests a closer combination between mercurial and bacterium and the necessity of having a mercury "neutralizer," such as a sulfur compound, in the subculture medium. Sodium thioglycollate, as contained in the thioglycollate medium used, appears to be suitable for this purpose. Furthermore, the thioglycollate medium has the advantage of being suitable for the cultivation of a wide variety of organisms, including many of the anaerobic sporeformers.

SUMMARY AND CONCLUSIONS

1. Results were compared on the bactericidal activity of various mercurial preparations using (*a*) Shippen retransfer technique, (*b*) 100 cc. of plain broth for subculture, and (*c*) thioglycollate medium for subculture

2. The results indicate the advisability of using a "neutralizing" medium such as thioglycollate medium as a subculture for determining *in vitro* bactericidal dilutions of mercurial preparations.

3. The Shippen retransfer technique and the use of 100 cc. of plain broth for subculture gave falsely high-killing dilutions in several instances, since they were not effective in eliminating the high bacteriostatic properties of the mercurial preparations tested.

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A Study of Assay Methods for Methylene Blue*

By F. A. Maurina and Neulon Deahl

The perchlorate precipitation method for the assay of methylene blue was presented several years ago in an unpublished report of work that had been conducted in this laboratory.[1] A subsequent study of the method was made by collaborating laboratories which eventually led to its inclusion in the U. S. P. XII.

It is the purpose of this paper to describe the results of our investigation. Data are presented here for certain analytical characteristics of various procedures that were tried during this investigation, some of which were not included in the earlier work.

* Received May 22, 1943 from the Analytical Laboratories of Parke, Davis & Company, Detroit, Mich.

[1] A. PH. A. meeting, 1937.

EXPERIMENTAL

The iodine absorption method of Morraw (1) has been the most commonly accepted procedure for the assay of methylene blue and is fairly accurate provided that most careful precautions are observed in the manipulative technique. He used acetic acid, while others used sodium acetate as a "means of stabilizing the reaction." Acetic acid, it is claimed, fixes the reaction as though the molecule of methylene blue absorbs 5 atoms of iodine while sodium acetate permits the absorption of 6 atoms of iodine. Holmes (2) investigated this reaction of methylene blue, as well as the reaction of several other basic dyes with iodine, and found that invariably the quantity of iodine taken up by the dye was dependent upon the concentration of iodine in the solution with which the precipitated dye-iodine complex was in equilibrium. Consequently, the reaction involved is not that of the formation of a complex of definite atomic proportions. Dealing

more specifically with the reaction as applied to the quantitative estimation of methylene blue, he found that for accurate work the method of Morraw (1) must be calibrated for variations in dye concentration.

A similar disparity exists when sodium acetate is used as the "stabilizer," as can be seen by examining the results of our work as given in Table I. The influence of variation in dye concentration is well defined.

TABLE I.—THE INFLUENCE OF THE VARIATION IN DYE CONCENTRATION UPON THE SODIUM ACETATE-IODINE ABSORPTION METHOD[a]

No.	Anhydrous Dye, Gm.	0.1 *N* Iodine Absorbed, Cc.	$C_{16}H_{18}N_3ClS$ Based on Absorbed Iodine, Using Factor 0.005328, %
1	0.0556	11.51	110.3
2	0.0769	15.39	106.6
3	0.1211	22.37	98.4
4	0.2035	34.74	91.0
5	0.2516	39.96	84.6
6[b]	0.0560	...	115.0
7	0.0900	...	101.1
8	0.0910	...	100.1
9	0.1000	...	95.2
10	0.1022	...	97.0
11	0.1028	...	98.4
12	0.1100	...	96.0
13	0.1514	...	88.6
14	0.2024	...	85.0

[a] Essential provisions of the U. S. P. XI method were followed.

[b] Results Nos. 6 to 14 were taken out of the correspondence from another laboratory. They used an entirely different sample of methylene blue and added 150 cc. of 10% sodium acetate instead of 50 cc.

Other factors contribute to the inconsistency of the iodine absorption method. For example, a given sample of methylene blue assayed 4.5% higher at 40° C. than at 25° C. Some observers report that iodine is absorbed by the filter paper during filtration. Accordingly Wales (3) advises filtering through glass wool or asbestos, while Sabalitschka and Erdmann (4) claim that filtering is unnecessary. Our own observations showed a loss of 1.1 cc. of 0.1 *N* iodine during filtration when the assay was carried out in the presence of sodium acetate but that no such loss occurred in the presence of acetic acid. This indicated a volatilization of iodine from solution rather than absorption by the filter paper and tests with moist starch paper held over the reaction flasks proved this point.

Obviously, the iodine absorption method is one that may be affected by the slightest variation in technique. Unless it is rigidly standardized there will exist a continued controversy between purchaser and supplier, and official investigator. For this reason it seemed advisable further to investigate the methods for analyzing methylene blue.

Few compounds are better adapted or more inviting to the analyst for study than methylene blue. It takes part readily in a number of quantitative reactions and it was our purpose in this work to study these reactions with the thought in mind of selecting one less likely to be affected by varying conditions than the iodine absorption method. Early in this work we found the perchlorate precipitation method which in our opinion is much superior to the iodine absorption method both in convenience and in accuracy. Consequently our researches are not as complete as we had originally planned, but, as it seems unlikely that we will have an early opportunity of extending this study, it would appear best to publish the results to date.

Although both the zinc chloride double salt (technical) and the hydrochloride (medicinal) are known as methylene blue, only the latter is known as the U. S. P. product, or tetramethylthionine chloride ($C_{16}H_{18}N_3ClS \cdot 3H_2O$), and will be dealt with here.

The reduction method with titanium chloride (18) where methylene blue is quantitatively converted into the colorless leuco compound has been generally accepted as the standard method of assay but for the average laboratory it is much too cumbersome. Because of the difficulty of obtaining ferric ammonium sulfate with the correct iron content to standardize the titanium chloride solution, other methods of standardization have been proposed (5, 6, 7, 8, 9). However, reduction methods in general are inconvenient because they must be conducted in an atmosphere of carbon dioxide which requires special apparatus.

REACTIONS STUDIED

By referring to its formula it is seen that the molecule of methylene blue contains 3 atoms of nitrogen and 1 each of chlorine and sulfur. Any single one of these elements can be determined quantitatively and the results of the analysis used to calculate the purity of the methylene blue. Sulfur can be determined as sulfate after fusion, nitrogen by the Kjeldahl method and chlorine by either the silver chloride gravimetric method or the Volhard method.

Throughout this entire study the same sample of methylene blue was used. It was a good grade of methylene blue which showed a moisture content of 12.39% and assayed 101.4% by the titanium titration method.

SULFUR DETERMINATION BY THE CARBONATE FUSION METHOD

Method.—Take a 0.3 to 0.4-Gm. sample which has previously been dried at 110° C., add 4 Gm. of a mixture of equal parts of anhydrous sodium and potassium carbonates, heat gently at first and then to complete fusion for 30 min. To avoid sulfur contamination from a gas flame the fusion may be conducted by closely fitting the crucible into a hole cut into a piece of heavy asbestos board so that one-third of the crucible projects above the asbestos, and heating the lower portion of the crucible by means of a large Bunsen or Meker burner. Cool, dissolve the melt in water, add about 5 cc. of bromine water, acidify with HCl, filter, wash the paper with a little water, dilute the filtrate and washings to about 200 cc., heat to boiling, and slowly add an

excess of barium chloride solution. Let stand overnight, filter through a Gooch crucible, wash free from chloride, dry, ignite, cool and weigh. Run a blank determination for sulfur in the fusion mixture.

Weight of $BaSO_4 \times 1.3698 = C_{16}H_{18}N_3ClS$

Results.—The method has been reported[3] to give satisfactory results (97.7%) when using the electric muffle furnace for fusion. With a gas flame, however, our results were low (97–82%), thus confirming the results reported by Morraw (1).

NITROGEN DETERMINATION

(*a*) *Kjeldahl-Gunning Method.*—Place 0.4 to 0.5 Gm. methylene blue, which has been previously dried at 110° C., in a digestion flask. Digest with 10 Gm. K_2SO_4, 25 cc. of concentrated H_2SO_4 and 0.3 Gm. crystallized copper sulfate until colorless and continue the heating for 2 hr. longer to complete the oxidation.

After cooling, dilute with about 200 cc. of distilled water. Next add sufficient sodium hydroxide solution to make the reaction strongly alkaline and complete the distillation of ammonia into 50 cc. of 0.1 *N* H_2SO_4 in the usual manner. Titrate the excess acid with 0.1 *N* alkali, using methyl red indicator.

1 cc. 0.1 *N* H_2SO_4 = 0.01066 Gm. $C_{16}H_{18}N_3ClS$

Results.—Four determinations gave, respectively, 99.57, 98.94, 99.23 and 98.72%. Although this method is not so rapid as the iodine absorption method of assay, it is more accurate and gives reproducible results.

(*b*) *Hydrogen Peroxide Digestion Method.*—Place 0.4 to 0.5 Gm. methylene blue, which has been previously dried at 110° C., in a digestion flask. Add 25 cc. of concentrated H_2SO_4 and follow this with 10 cc. of hydrogen peroxide (30%), poured in slowly along the side of the flask. Heat to SO_3 fumes, cool slightly, add 5 cc. more of hydrogen peroxide (30%) and again heat to SO_3 fumes for 2 min. Allow the colorless digested mixture to cool, dilute with about 200 cc. of water and proceed with the distillation of ammonia exactly as in (*a*).

Results.—Four determinations gave respectively, 99.10, 98.75, 98.67 and 99.32%. The method is accurate, convenient and rapid. Oxidation of the sample is completed within a few minutes with apparently no loss in accuracy. Compared to the Kjeldahl-Gunning method which requires 2 or 3 hr. for complete digestion, it represents quite a saving in time.

CHLORINE DETERMINATION

The reaction of methylene blue with potassium perchlorate, thus, $C_{16}H_{18}N_3SCl + KClO_4 = C_{16}H_{18}N_3SClO_4 + KCl$, forms an excellent basis for the accurate determination of the chlorine content. Bolliger (10) suggested this method but made no study of it. Although no chloride is precipitated with silver nitrate even on long standing (11), the measurement of the conductivity has shown that in solution, methylene blue hydrochloride is electrolytically dissociated (12). Moreover, the chlorine goes quantitatively into the filtrate after separation of the precipitated methylene blue perchlorate.

Based upon these facts the chlorine content of methylene blue is easily determined by the following method.

Method.—Place about a 1.0-Gm. sample of methylene blue, accurately weighed, *before drying*, into a 500-cc. volumetric flask and dissolve in about 200 cc. of water. Add to this 4 Gm. of $KClO_4$ and shake well to precipitate all of the methylene blue as the perchlorate, add 50 cc. of 0.1 *N* $AgNO_3$ and 2 cc. of concentrated HNO_3, make up to volume with water and shake well. Filter through a dry filter paper, reject the first 20–30 cc. of filtrate and titrate a 200-cc. aliquot portion of the remaining filtrate with 0.1 *N* KCNS, using ferric ammonium sulfate test solution as indicator.

1 cc. of 0.1 *N* $AgNO_3$ = 0.03198 Gm. $C_{16}H_{18}N_3SCl$

Correct the weight of the sample for the loss at 110° C. in order to obtain the anhydrous weight.

Results.—Six analyses gave the following results, 99.54, 99.94, 99.40, 99.63, 100.05 and 99.51%. The results are consistent but in order further to test the method, assays were run on several different samples of methylene blue that were lower in purity than the standard sample used for this investigation. In all cases, equally as concordant results were obtained.

TABLE II

Weight of Sample, Gm.	Weight of $KClO_4$ Used, Gm.	0.1 *N* $AgNO_3$, Cc.	Reaction Heated	HNO_3, Cc.	Time Allowed for Reaction before Filtering, Min.	%
1.0	4	50	No	1	5	99.5
0.5	4	50	No	1	30	99.7
1.0	4	50	No	1	60	99.3
0.5	2	50	No	2	5	99.1
1.0	2	50	Yes	2	5	99.7
0.5	2	50	Yes	2	5	98.9

Remarks.—Because of the consistent results given by this method we undertook a more extensive study particularly in regard to the influence of those factors which affect the iodine absorption method of assay. The conditions of the assay were varied as recorded in Table II. A study of the

[3] Correspondence.

condensed tabulation will show that, unlike the iodine absorption method, this reaction is not influenced by variations in factors such as dye concentration, acidity, temperature and time. It is not necessary, therefore, for each analyst to calibrate the method for himself.

Silver nitrate does not react with methylene blue perchlorate and can be added directly to the flask containing the precipitate without introducing an error. This was confirmed by a trial assay in which a Volhard determination was run on a measured portion of the filtrate, after removing the methylene blue perchlorate.

The filtrate from the methylene blue perchlorate-silver nitrate mixture possesses a bluish tint which may interfere with the sulfocyanate titration, but this can be held at a minimum by using an excess of potassium perchlorate in the assay. The influence of using only 2 Gm. in the assay is shown in Table II.

Moisture in Methylene Blue.—It must be emphasized here that the reaction used in this assay is applicable only in the case of undried methylene blue. Attempts to apply the method to the dried material failed. Either the reaction does not go to completion or other dye substances are formed during the drying of methylene blue that fail to react with potassium perchlorate. This is evidenced by the large amount of dye that remains in solution. Even after standing for several hours in contact with an excess of potassium perchlorate the filtrate is highly colored.

Other investigators in attempting to dry methylene blue have reported an evidence of decomposition. Wales and Nelson (13) found that dyes often held absorbed water more firmly than so-called water of crystallization and that it is not always possible to determine either by loss in weight on heating. Atack (12) found that at 105° C. water of crystallization is not completely expelled and that at 110° C. there is a rapid continuous loss with evolution of a substance having an intensively disagreeable odor. The residue gives a pale lilac extract with ether, not given by the original sample, which probably is due to partial conversion into methylene azure. A soluble green substance is often formed in the residue which is not reducible even with a large excess of titanium chloride. Morraw (14) found that samples from different manufacturers varied widely in water content which seems to support the theory that methylene blue does not contain figure 3 molecules of water of crystallization, as assigned to it by the U. S. P. He found marked decomposition at 130–140° C. and that the moisture requirements of the U. S. P. were best met by heating at 110° C. In our own work we have standardized the method for determining moisture at 110° C. for 18 hr.

It is necessary, therefore, in the method for determining chlorides, as we have described it above, to correct the weight of the sample by deducting moisture (loss in weight at 110° C.).

It would seem from the work presented so far that methods for determining single elements within the molecule of methylene blue are convenient methods for the assay of this substance, particularly in the case of nitrogen and of chlorine. But unfortunately these methods fail to detect the presence of the element if it is there as an impurity or as an adulteration and the methods are useful only in so far as they determine the total amount of element. Since it is difficult to determine soluble salts of chlorine or nitrogen in methylene blue, the application of these methods is limited and for precise work they would naturally be unreliable.

The property of methylene blue to combine with many substances to form a compound of definite composition opens a wide field of reactions for analytical study. The reaction with iodine, of course, belongs to this class, but the errors involved here have already been discussed. Picric acid (10, 15), as well as other alkaloidal precipitants, form well-defined compounds with methylene blue. Certain inorganic acids react with methylene blue to form precipitates, some of which have already been studied and proposed for analytical purposes (12, 16, 17). Of this group of reactions 2 have proved, in our hands, to be the most suitable for analytical adaptation and will be discussed here.

REACTION WITH PICRIC ACID

1. Volumetric Assay.—Based upon the fact that methylene blue picrate is only slightly soluble in aqueous solution but soluble in chloroform, Bolliger (10) devised a scheme for titrating methylene blue in a separatory funnel, until finally after shaking with chloroform the aqueous solution is colorless. In our own trials we found that the end point is too uncertain for accurate work.

Since there are few who would not prefer a volumetric scheme of analysis to a gravimetric we made further attempts to titrate methylene blue with picric acid. It seemed to us that it should be possible to determine an excess of picric acid by titration with alkali, so the following experiment was tried.

A solution of hydrous methylene blue was prepared so that 25 cc. was equivalent to 0.1 Gm. of anhydrous dye. Standard 0.01 *N* picric acid was prepared by dissolving 2.29 Gm. in a liter of water and titrating this with 0.01 *N* sodium hydroxide, using methyl red indicator. For the assay an excess of standard picric acid solution was added to a measured volume of methylene blue solution and the excess determined in an aliquot portion of the filtrate from the precipitated methylene blue picrate, by titrating with 0.01 *N* sodium hydroxide using methyl red indicator. The end point is sharp and distinct, but further investigations showed that, just as in the iodine absorption method, the results are influenced by variations in the dye concentration. In Table III we have recorded the results of this experiment. It will be noted that the amount of picric acid consumed is proportional to the excess present in the assay. It is possible that by standardizing the conditions in this assay, results may

be obtained that are equal to those of the iodine absorption method in accuracy.

TABLE III

Sample Taken, Cc.	0.01 *N* Picric Acid Added, Cc.	Size of Volumetric Flask, Cc.	Time Allowed for Reaction	Aliquot Titrated, Cc.	0.01 *N* Picric Acid Consumed, Cc.
25	50	100	10 min.	50	25.37
25	50	100	10 min.	50	25.17
25	40	100	10 min.	50	20.09
25	40	100	10 min.	50	20.17
25	50	500	16 hr.	100	26.35
25	50	500	16 hr.	100	26.87

2. Gravimetric Assay.—Methylene blue picrate is formed by a direct molecular combination of methylene blue and picric acid without the usual contamination that accompanies precipitates formed by double decomposition. It would seem then that the precipitate needs to be washed with but a little water in order to free it from the excess picric acid. François and Seguin (15) tried this scheme and obtained excellent results after washing the precipitate with only 10 cc. of water and drying in air. In our hands the method was not so successful. We have shown that the influence of variations in washing is well defined which indicates a greater solubility for methylene blue picrate than is usually accredited to it.

REACTION WITH PERCHLORATE

1. Volumetric Assay.—The adaptation of the reaction between methylene blue and potassium perchlorate for the volumetric estimation of chlorine in methylene has already been discussed.

2. Gravimetric Assay.—Methylene blue gives with either potassium or sodium perchlorate a quantitative yield of methylene blue perchlorate, which in our opinion forms the basis of a most accurate method for standardizing the dyestuff—more accurate and more reliable than any other method presented thus far. The reaction was used by Monnier (16) to detect perchlorates in salt peter. Fedarova (17) also described an insoluble compound of methylene blue perchlorate. Atack (12) studied the properties of methylene blue perchlorate along with those of several other precipitates formed by the interaction of methylene blue with inorganic acids and found that the perchlorate was the only salt stable enough for quantitative work. His scheme of analysis included washing the precipitate with water followed by an alcohol-ether mixture. He pointed out, however, that due to hydrolysis of the precipitate on filtering, the results of the method were likely to be low.

The work of these investigators prompted us to a further study of this precipitate, especially in regard to its solubility. Various wash solutions were tried. Water, it seems, is the least satisfactory because it permits the precipitate to hydrolyze back to the very soluble methylene blue. Alcohol acts in a similar way. A mixture of 9 parts of ether to 1 part of alcohol gives fairly good results. The most satisfactory wash solution, however, was found to consist of a very dilute solution of the same salt used as the precipitant. Even with large quantities of this wash solution the precipitate undergoes very little hydrolysis as can be witnessed by the almost colorless filtrate.

In our experiments we tried both the sodium and the potassium perchlorate. Although quantitative results were obtained with either salt, the former, because of its greater solubility, is more easily washed from the precipitate and gave the most concordant results. Sodium perchlorate, therefore, was the precipitant used in the experiments reported here.

In order to test the dependability of the method it was subjected to varying conditions and the effects on the results measured. The general procedure for the assay was to precipitate a solution of 0.1 Gm. hydrous methylene blue in 100 cc. of water with 0.5 Gm. of sodium perchlorate, filter through a Gooch crucible, wash and weigh the dried precipitate.

In Tables IV and V we have recorded in condensed form the results of our tests. Note the consistency of the results where 0.1% sodium perchlorate was used as the wash solution; also the uniformity of results whether 50 cc. or 100 cc. of wash solution is used.

TABLE IV.—THE INFLUENCE OF DIFFERENT WASH SOLUTIONS UPON METHYLENE BLUE PERCHLORATE

Sample Taken for Assay, Gm.	Wash Solution Used	Results, %
0.1	50 cc. of 0.1% $NaClO_4$ soln.	99.48
0.1	50 cc. of 0.1% $NaClO_4$ soln.	99.00
0.1	50 cc. of 0.1% $NaClO_4$ soln.	99.38
0.1	100 cc. of 0.1% $NaClO_4$ soln.	99.42
0.1	100 cc. of 0.1% $NaClO_4$ soln.	99.53
0.1	100 cc. of 0.1% $NaClO_4$ soln.	99.44
0.1	10 cc. of mixt. of 9 parts ether to 1 part alcohol	98.81
0.1	25 cc. of mixt. of 9 parts ether to 1 part alcohol	99.19
0.1	25 cc. of mixt. of 9 parts ether to 1 part alcohol	98.79
0.1	100 cc. of mixt. of 9 parts ether to 1 part alcohol	99.19
0.1	100 cc. of mixt. of 9 parts ether to 1 part alcohol	95.55
0.1	100 cc. of ether	99.79

From the results recorded in Table V it can be concluded that the effect of slight variations in acidity is negligible. Moreover, we found that sodium acetate can be used in this assay for the purpose of adjusting the acidity without influencing the results.

TABLE V.—THE INFLUENCE OF VARYING THE ACIDITY UPON THE METHYLENE BLUE PERCHLORATE REACTION

Sample Taken for Assay, Gm.	Amount of 10% HCl Added, Cc.	pH of Solution	Gm. $NaClO_4$ Used for Precipitating	0.1% $NaClO_4$ Wash Solution Used, Cc.	Results, %
0.1	0	4.65	0.5	50	99.48
0.1	0	4.65	0.5	100	99.38
0.1	2	3.15	0.5	50	99.58
0.1	5	0.90	0.5	50	98.80
0.1	10	..	0.5	50	98.40
0.1	10	..	0.5	50	98.90

Further experiments showed that variations in time, temperature and size of sample, in fact, variations in any one of the factors that were found to influence the iodine absorption method of assay, had little or no effect upon the perchlorate gravimetric method. The precipitate of methylene blue perchlorate was dried at 110° C. for 24 hr. without any signs of decomposition, but drying for 1 hr. at this temperature is sufficient to bring the precipitate to constant weight. The procedure is extremely simple, with a minimum of precautions necessary to obtain good results. Although the advantages of this method are perfectly obvious, it is important to note that in this assay we are actually measuring a compound of methylene blue that has a definite identity. In this respect it represents an accurate evaluation of the purity of methylene blue. We are recommending the methylene blue-perchlorate gravimetric method as a standard procedure for the assay of methylene blue.

METHOD RECOMMENDED

Dissolve about 0.1 Gm. of undried methylene blue, accurately weighed, in 100 cc. of distilled water in a 250-cc. beaker, using gentle heat to complete the solution if necessary. Add to this a solution of 0.5 Gm. of sodium perchlorate in 30 cc. of distilled water and stir the mixture intermittently for about 10 min., in order to complete the precipitation. Filter through a prepared Gooch crucible which has previously been dried at 110° C. and weighed. With the aid of a rubber-tipped glass rod transfer the last traces of precipitate from the sides of the beaker to the crucible, using 0.1% sodium perchlorate in distilled water for washing. Finally wash the precipitate with an additional 50 cc. of wash solution, dry the crucible and precipitate for 1 hr. at 110° C., cool and weigh.

One gram of methylene blue perchlorate is equivalent to 0.8333 Gm. of methylene blue, anhydrous.

Correct the weight of the sample for the moisture content, determined on a separate portion of methylene blue by heating at 110° C. for 18 hr.

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Notes on Modifications of the Werner Method for the Determination of Sulfonamides*

By S. W. Lee and N. B. Hannay†

The original Werner method (1) for the determination of sulfanilamide and its derivatives is being rather widely used, especially abroad. Compared with the Bratton and Marshall method (2), it has the advantage of simplicity. Critical studies of the Werner method however, have, shown it to be subject to inaccuracies because of variations in the intensity of the final color, as well as that of the reagent, with slight changes in acid concentration, and because of the tendency of the colored compound to crystallize out at high-sulfonamide concentration. The influence of both these causes of error is more marked in the determination of "total" sulfonamide levels. In regard to this, Morris (3) has stated that "the values of acetylsulfanilamide were so erratic as to be worthless." Morris was of the opinion that it was necessary to avoid the use of trichloracetic acid because of its variable decomposition on heating in aqueous solution. He based a method on the Werner reagent (*p*-dimethylaminobenzaldehyde) in which toluene sulfonic acid and a buffering system were used. He also substituted a 2% alcoholic solution of the Werner reagent for the older acidified aqueous solution.

Churg and Lehr (4) claim that by substituting an alcoholic solution of the Werner reagent for the acid solution, and by working in a system containing one-third alcohol by volume, they can eliminate the sources of error. A study of this and related modifications has been carried out in this laboratory.

EXPERIMENTAL

Method of Measuring Colors.—The densities of all solutions were measured against reagent blanks which were prepared in the same way as in a determination, except that they contained no sulfonamide. A Coleman Universal spectrophotometer was used in all determinations with the wavelength dial set at 450 mμ, the peak of absorption of the anil which is formed from the aldehyde and the amino sulfonamide. This instrument is, of course, sensitive to variations in the intensities of yellow colors, and allows accurate determinations. Cuvettes (20 x 40 mm.) were used, the longer cell length for lower concentrations.

The absorption curve of the *p*-dimethylaminobenzylidine-*p*-aminobenzenesulfonamide which is formed is indicated in Table I.

TABLE I.—ABSORPTION OF *p*-DIMETHYLAMINOBENZYLIDENE-*p*-AMINOBENZENESULFONAMIDE

Wave Length, mμ	Color Density
400	0.037
425	0.059
450	0.070
475	0.059

Effect of Alcohol Content on the Color Density.—All these determinations were made in a volume of 15.0 ml. and each contained the same weight of sulfanilamide. The variation of density reading with alcohol content is shown in Table II, each color being measured against its related reagent blank.

TABLE II.—EFFECT OF ALCOHOL ON THE COLOR DENSITY

Alcohol Added, Ml.	Alcoholic Werner Reagent, Ml.	*p*H	Color Density
1	1	0.5	0.29
3	1	...	0.34
5	1	0.6	0.30
7	1	...	0.23
10	1	0.8	0.13

The Effect of Variation of Hydrogen-Ion Concentration on the Color Density.—The color densities of solutions containing a constant amount of sulfonamide, color-forming reagent, and alcohol, but made up at various acidities were measured. Citric acid-disodium phosphate buffers were used to bring the *p*H to 2.2. Varying amounts of trichloracetic acid

TABLE III.—EFFECT OF *p*H ON THE COLOR DENSITY

*p*H	Trichloracetic Acid 15%, Ml.	Color Density
5.0	0	0.0
3.8	0	0.02
3.0	0	0.03
2.2	0	0.16
1.5	0.1	0.12
1.2	0.4	0.24
0.95	1.2	0.24
0.85	1.5	0.22
0.77	2.0	0.22

* Received May 21, 1943, from the Wallace Laboratories, New Brunswick, N. J.

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were used for more acid solutions, and their *p*H values determined with a Leeds and Northrup *p*H meter. The final volume of the solutions was again 15.0 ml., and the comparisons were made against corresponding reagent blanks.

The Effect of Amount of Reagent on the Densities Obtained.—Varying amounts of 1% alcoholic solution of *p*-dimethylaminobenzaldehyde were used in a series of tubes containing constant amounts of sulfonamide and alcohol, kept at very nearly the same *p*H. The results are shown in Table IV. This same experiment was performed, with a second sample of *p*-dimethylaminobenzaldehyde, and the amount needed for maximum density was then found to be different. In this case, the aldehyde gave an almost colorless solution. The density produced from a constant amount of sulfonamide reached a maximum when 2 ml. was used, and the value remained constant to 5 ml. Volumes were made to 15.0 ml. with distilled water. Trichloroacetic (2 ml., 15%) was added in all trials.

TABLE IV.—EFFECT OF AMOUNT OF ALCOHOLIC REAGENT ON THE COLOR DENSITY

Alcoholic Reagent 1%, Ml.	Alcohol, Ml.	*p*H	Color Density
0.1	4.9	0.4	0.11
0.5	4.5	0.5	0.40
1.0	4	0.6	0.50
1.5	3.5	...	0.48
2.0	3	0.7	0.35
5.0	0	...	0.10

Test Analyses on Blood Samples.—Procedure: Oxalated whole blood (1.00 ml.) was added dropwise with shaking and stirring to 9.0 ml. of 3.33% trichloracetic acid (2.0 ml. of 15% trichloracetic and 7.0 ml. water). The precipitated proteins were filtered on Whatman No. 42 paper, and 6.0 ml. of the filtrate used for analysis. Trichloracetic acid (0.8 ml., 15%) and the alcoholic *p*-dimethylaminobenzaldehyde (1.0 ml., 1%) were added. The volumes were made up to 15.0 ml. with distilled water, and the colors formed compared against reagent blanks. This is an adaptation of the Churg and Lehr procedure.

Results: In the determination of free sulfonamides, the results were accurate only when blank readings (before medication or addition of drug) were taken. It was found that blood samples which did not give appreciable readings with the Bratton and Marshall method, gave levels up to 2 mg. per 100 cc. with this procedure. The blood blank readings were additive, and the recovery of added sulfathiazole was high. This is shown in Table V. In this experiment, blood was taken from 6 persons who had received no sulfonamide. Blanks were run on the samples by 2 methods and sulfathiazole was added at 2 levels for the purpose of studying recovery by the adapted Churg and Lehr method.

TABLE V.—RECOVERIES OBTAINED BY THE CHURG AND LEHR ADAPTATION OF SULFATHIAZOLE ADDED TO BLOOD

Sample	Bratton and Marshall Blank, Mg. %	Churg and Lehr Blank, Mg. %	Churg and Lehr 2 Mg. %	Churg and Lehr 4 Mg. %
1	0.2	0.8	2.8	5.0
2	0.15	1.3	3.2	4.8
3	0.15	1.6	4.1	6.0
4	0.1	1.6	...	6.1
5	0.2	2.3	4.3	6.6
6	0.1	2.3	4.4	6.6

Blank readings were often found with human, horse and rabbit blood, though not always.

In the determination of total sulfonamides, it was impossible to obtain accurate results. Our findings were in accord with those of Morris, and the inaccuracies again due to small changes in acidity. Using the exact method of Churg and Lehr, and making measurements spectrophotometrically, it was not found possible to obtain the constant factor 1.15, which they use to compensate for the color decrease due to the acid liberated during the hydrolysis step. This "constant" was found to vary over a wide range, depending on the shape of the vessel used for hydrolysis. The value was often 2, and in some cases, even more. The values obtained, except under extremely standardized conditions, were inaccurate. Attempts were made to use buffers to give a satisfactory control of the *p*H, but this did not prove satisfactory. It was not possible to determine the *p*H accurately with the indicator quinaldine red, which changes from red to colorless in the optimum range of *p*H 2 to 1. The same conclusions were drawn from a study of the similar problem using the adapted Churg and Lehr procedure.

SUMMARY

1. An adaptation of the method of Churg and Lehr has been found to give accurate analyses for "free" sulfonamides, provided allowances are made for ostensible levels of the body fluid under investigation prior to medication (blood blanks).

2. It has not been possible to obtain accurate results on "total" sulfonamides, using either the method of Churg and Lehr or the adaptation of it.

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Studies on the Barbiturates

XXVI. The Use of Lloyd's Reagent in the Quantitative Estimation of Barbiturates in the Urine*

By Melvin W. Green, Fletcher P. Veitch and Theodore Koppanyi†

The estimation of barbiturates in urine by the Koppanyi test as originally developed may be rendered difficult by the presence of chromogens in chloroform or ether extracts of urine which tend to mask the color developed, and by the actual reaction of the cobalt reagent with certain other substances, to produce colored complexes. These facts have long been recognized by Koppanyi and co-workers (1) and lately have been noted by other observers (4, 5).

This investigation was undertaken with the purpose of producing chloroform or ether extracts of urine free from chromogens, and to show how reactions due to substances other than barbiturates may be eliminated.

EXPERIMENTAL

Absorption Characteristics of the Barbital-Cobalt Color Complex.—Recrystallized barbital was dissolved in chloroform and this solution treated with cobaltous acetate and isopropylamine in the usual fashion. The resulting colored solution was examined in a Coleman regional spectrophotometer, model 10-S, employing a round cell approximately 10 mm. in diameter, and a 30-mμ slit. Distilled water was used as a blank. The barbital-cobalt complex exhibited maximum absorption at 560 mμ (see Fig. 1). Kraus and Riley (5) showed that the amytal-cobalt complex showed maximum absorption at 565 mμ.

Removal of Chromogens from the Urine by the Use of Lloyd's Reagent.—Experimentation with various adsorbents for the removal of chromogens from urine led us to adopt the following procedure. The urine to be examined was shaken in a flask for 10 min. with 2 Gm. of Lloyd's reagent (a purified siliceous earth) and 5 cc. of 5% sulfuric acid for each 20 cc. of urine used, and then filtered. If the urine was highly colored by blood pigments, precipitation with tungstate (7) was resorted to prior to the adsorption on Lloyd's reagent. A convenient aliquot of the filtrate was removed and shaken with 10 volumes of ether or chloroform in 10 equally divided portions in a separatory funnel. The combined extracts were evaporated to dryness and the residue dissolved in a convenient volume of chloroform. The cobalt and isopropylamine reagents (1, 2, 3) were then added and the color read in a visual colorimeter. In this manner the most highly pigmented human and animal urines, even those containing relatively high concentration of bile pigments or methylene blue, were rendered fit for colorimetry. All attempts to recover barbiturates from the Lloyd's reagent were unsuccessful, indicating that barbiturates were not adsorbed. Extracts of human urines, known to contain no barbiturates, when treated in this fashion showed only 2% transmission at 560 mμ after addition of the reagents, indicating that interfering chromogens are almost quantitatively removed. The remaining chromogens were completely removed by shaking the chloroform or ether extract with 50% sulfuric acid (see Table I). This treatment, however, was abandoned as some loss of barbiturates, either by solution or destruction, was observed.

TABLE I.—TRANSMISSION[a] AT 560 Mμ OF COBALT-ISOPROPYLAMINE ADDED TO HUMAN URINE EXTRACT

	Treatment of Urine	Transmission, %
1.	Chloroform extracted	85
2.	Urine + Lloyd's Reagent; extracted with chloroform	98
3.	Urine + Lloyd's Reagent; The chloroform extract shaken with 50% sulfuric acid.	100

[a] Determined with Coleman spectrophotometer.

Known quantities of the most commonly used barbiturates were added to human urine and recovered by the method described above. The results of these experiments are summarized in Table II. In all cases recorded in the table, barbital was the standard against which the other barbiturates were compared. We believe that for precise work it is better to use the specific barbiturate in question as a standard, although this is not always possible in chemical toxicology. The slight differences in color produced by some derivatives with the cobalt reagent and differences in rate of extraction may account, at least in part, for the magnitude of the standard errors.

Adaptation of the Cobalt-Isopropylamine Test to Spectrophotometric Analysis.—Various concentrations of pure barbital in chloroform were analyzed

* Received June 16, 1943, from the Departments of Pharmacology and Materia Medica and Biochemistry, Georgetown University, School of Medicine, Washington, D. C.

† The authors are grateful to Mr. J. T. Lloyd of the J. T. Lloyd Laboratory of Cincinnati and to Mr. J. K. Lilly of the Eli Lilly Company of Indianapolis for supplying the Lloyd's reagent.

TABLE II.—RECOVERY OF ADDED BARBITURATES FROM URINE

Barbiturate Added	Concentration of Barbiturate Added, Mg./Cc.	Found, Mg./Cc.	Recovery, %	Standard Error
Barbital	1.0	0.983	98.3	4.3
Phenobarbital	1.0	1.038	103.8	1.7
Isobutylallyl barbituric acid (Sandoptal)	1.0	0.931	93.1	0.9
Cyclohexenylethyl barbituric acid (Phanodorn)	1.0	1.013	101.3	4.7
Pentobarbital (Nembutal)	1.0	0.971	97.1	4.8
n-Butylethyl barbituric acid (Neonal)	1.0	0.958	95.8	1.2

in the spectrophotometer at 560 mμ and the per cent transmittance plotted against the concentration in mg./100 cc. It will be noted that the color developed in the test obeys Beer's law in concentrations up to 30 mg./100 cc. (Fig. 1). For higher concentrations, the barbital may be either approximated from the curve or the chloroform solution

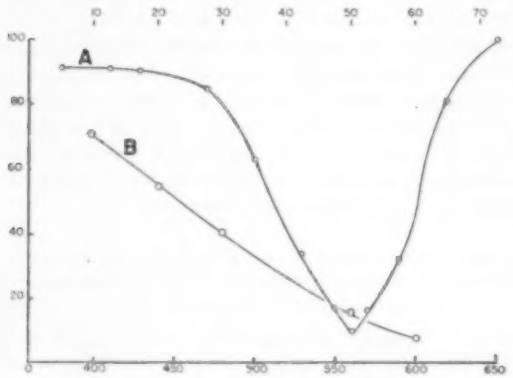

Fig. 1.—Spectral Analysis of Barbital with the Cobalt-Isopropylamine Reagent.

Curve A, spectral curve of barbital. Abscissa—wave length in mμ; ordinate—per cent transmission.

Curve B, concentration = transmission curve. Abscissa—concentration of barbital in mg. per 100 cc.; ordinate—per cent transmission.

may be carefully diluted to fall within the lower range where the determination can be made more precisely. The quantitative recovery of barbital from urine was then attempted using the above-mentioned procedure of extraction and determining the percentage recoveries by spectrophotometric methods. Using a single extraction with 10 volumes of chloroform, the recovery was only 87%. If the extraction was made with the same total volume of chloroform in divided portions, the recovery was better, but not completely satisfactory. This is to be expected when it is realized that the solubility of barbital in water is 1 part in 130 and the solubility in chloroform is 1 part in 75. Since the solubility of barbital in ether is 1 part in 35, 20 volumes of ether in portions of 10 cc. each were used to extract the drug from the treated urine. The ethereal extract was then evaporated to dryness and the residue dissolved in a convenient volume of dry chloroform whereupon the recovery rose to about 95%.

DISCUSSION

It has always been recognized by Koppanyi and co-workers (2, 6) that certain substances such as guanidine, theophylline, sulfonamides, acetates, etc., give positive tests with the cobalt reagent. Other chemicals such as biuret and oxamide which give a positive test are not extracted by chloroform. Substances like guanidine and creatinine give positive responses in alcoholic media only. The behavior of sulfonamides in the cobalt color tests for barbiturates has been discussed in a previous communication (6).

In order to test the assumption that urines may yield false positive values, 41 urines from pathological cases of the Georgetown Hospital and 14 urines from patients receiving medication other than barbiturates were examined by means of the barium, lithium and the isopropylamine tests. In no instance was the test positive unless the patient received barbiturate medication. In other words, when the urine contained appreciable amounts of acetone bodies, glucose, large amounts of female sex hormones, albumin, blood, and drugs and their metabolic degradation products, such as cascara, methenamine, ephedrine, methylene blue and others, the cobalt color tests were still negative. Eight dog urines and four cat urines were also examined and found to be negative in spite of the fact that they were unusually heavily pigmented.

The clinical interpretation of results based on quantitative recovery of barbiturates is not always easy. It is quite within the realm of possibility, particularly in the case of barbiturates which are destroyed to a large extent by the tissues, that the characteristic color is not due to unchanged barbiturate, but to some degradation product, probably containing an intact imidazol grouping. Consideration should be given to the possibility that these fragments might even possess hypnotic activity. Biological testing is therefore of little value unless the substance having hypnotic activity is actually identified by isolation.

In nephropathic subjects, additional care is needed in the interpretation of quantitative results. Argy, Linegar and Dille (8) have shown that while a normal subject excreted approximately 10% of a given dose of barbital in 24 hr., nephropathics excreted but 6% on the average during the same interval. This indicates that in the presence of kidney damage, one must be exceedingly wary about correlating the amount of a barbiturate found in the urine with the amount probably taken. Furthermore, it is possible that in the case of kidney damage where the urines were found to be negative, the patient may have actually taken small amounts of barbiturates, but the damage to the

kidney may have raised the threshold beyond the limits of the test. In one case of seconal coma where the patient recovered and confessed to having taken a large amount of this drug, we could find no trace of barbiturate in the urine. The urine indicated an extraordinarily severe kidney damage, for it was loaded with blood, pus and albumin to the point of ropiness. Since only small amounts of seconal are normally excreted by the kidneys, renal damage might actually render it indetectable. Therefore, in cases of kidney pathology where urines respond negatively to the cobalt color tests, a test should be made on the blood before definitely excluding barbiturate poisoning.

SUMMARY

1. It has been shown that urines known not to contain barbiturates do not give the characteristic blue color in the Koppanyi test under the proper conditions.

2. The determination of barbiturates in the urine is rendered more precise by the use of Lloyd's reagent which adsorbs pigments and other chromogens without removing the barbiturates.

3. The cobalt-isopropylamine test has been adapted to spectrophotometric estimation of barbital, and it has been shown that the test obeys Beer's law in concentrations up to 30 mg./100 cc.

4. The following directions represent the most recent modification of the detection and estimation of barbiturates in the urine.

(*a*) Add 5 cc. of a 5% solution of sulfuric acid and 2 Gm. of Lloyd's reagent to 20 cc. of urine in an Erlenmeyer flask. Shake thoroughly for 10 min.

(*b*) Filter and shake an aliquot of filtrate with at least 10 volumes of ether in equally divided portions.

(*c*) Evaporate the combined ether extracts and dissolve the residue in a convenient volume of chloroform.

(*d*) Test the chloroform extract for barbiturates in a standard colorimeter or spectrophotometer using methods previously prescribed.

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(3) Linegar, C. R., Dille, J. M., and Koppanyi, T., *Ibid.*, 24 (1935), 847.

(4) Kraus, R. F., and Riley, R. F., *J. Pharmacol.*, 71 (1941), 287.

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(7) Hawk, P. B., and Bergeim, O., "Practical Physiological Chemistry," Tenth edition, Blakiston, Philadelphia, 1931, p. 411.

(8) Argy, W. P., Linegar, C. R., and Dille, J. M., *J. Pharmacol.*, 57 (1936), 258.

WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Street Chicago, Ill., seeks information on sources of supply for the following chemicals:

Bulbocapnine
Porphyrindien
5-Amino-nicotinic Acid
Tribromo Caffein
Caronic Acid (3,3-Dimethyl, 1,2-cyclopropane-decarboxylic acid). Either or both forms (*cis* and *trans*) will do
3-Acetoxy, 5,6-etiocholenic acid (I)
3-Hydroxy, 5,6-etiocholenic acid (II)
Methyl ester of 3-hydroxy, 5,6-etiocholenic acid (III)
Dextro-mandelic Acid
Laevo-mandelic Acid
Yeast Nucleic Acid
Ornithine 1 g.
Deca Tetra Enoic Acid
Myricin
β-Cyclocitral
Alpha-keto-glutaric Acid
Guvacin
Arecaidine

Book Reviews

Food and Drug Regulation, by STEPHEN WILSON with an introduction by WALTON H. HAMILTON, American Council on Public Affairs, 2153 Florida Avenue, Washington, D. C., 1942. xi + 177 pp., 15 x 23 cm. Price, cloth ed. $3.25; paper ed. $2.50.

Despite the direct relationship of food and drugs to public health, there is probably no other field of economic return in which the ancient slogan *caveat emptor* has been so thoroughly entrenched. Dr. Wilson, by invitation from the American Council on Public Affairs, has aptly and thrillingly told the story of the battle for legislative reform in these fields in his book, "Food and Drug Regulation."

A citation of the chapter headings will give the reader an indication of the ground covered by this brochure: Food and Defense, History of Food and Drug Legislation, The Federal Food and Drug Law, The Difficulties of Administration, New Legislation, The Food, Drug and Cosmetic Law of 1938, and Causes and Consequences.

In the first chapter, Food and Defense, Dr. Wilson points out that in the United States the problem of getting sufficient quantities of food has been substantially solved and that we are now in the phase where it is necessary to prevent perpetration of fraud on the customer and prevent the sale of food unfit for consumption. The next logical stage, which has presumably just begun, is characterized by the task of assuring the people of a diet properly balanced nutritionally.

To pharmaceutically minded readers, the last several chapters leading up to the advent of the Food, Drug and Cosmetic Law of 1938 and its consequences will be particularly enlightening. In these sections, the complexities of the pros and cons and the effects of pressure groups is especially well brought out. It is fortunate that Dr. Wilson appreciates the technological basis upon which enforcement must naturally rest. This is exemplified by his statement: "Fundamental changes in the economic system and technological developments in the industry have resulted in a rapid evolution—if not a revolution—in the methods of manufacture and distribution of foods and drugs. Increased consumer demand for more ready-to-serve foods, enlarged plant capacity for mass production, keener competition, new products, new processes, new sources for raw material—all of these factors have made the task more complex. The administration, with limited funds, must keep abreast of these rapid changes. There is no pause, no stopping place. The problem is dynamic, not static."

Although necessarily incomplete, the account of attempts to control the quality of food and drugs legislatively is thrillingly told in Dr. Wilson's book. It is a story told not in cold legalistic terms but with all of the warmth of an appreciation of the position of such regulation in an over-all social-economic pattern.

The book is well documented and the typography is above reproach. It is unfortunate that the book contains no index.—M. W. GREEN.

Organic Chemistry, by G. ALBERT HILL, Professor of Organic Chemistry, Wesleyan University, and LOUISE KELLEY, Professor of Organic Chemistry, Goucher College. The Blakiston Company, Philadelphia, Pa., 1943. viii + 919 pp. 16 figs., 71 tables, 15 x 23 cm. Price, $4.00.

This is not a revision of the textbook by the same authors published about ten years ago but is an entirely new and comprehensive volume designed to serve both as a textbook and as a reference book.

The book is divided into the usual divisions with 25 of its 46 chapters being devoted primarily to aliphatic compounds; 13 being devoted to aromatic compounds; and one (the introduction) being devoted to a theoretical discussion of the nature of atoms, chemical linkages and related material. The last seven chapters are devoted, respectively, to: (1) Dyes, (2) Alicyclic Compounds, (3) Terpenes and Camphors, (4) Heterocyclic Compounds, (5) Alkaloids, (6) The Steroid Group Including Plant Pigments and Vitamins, and (7) Polymerization. In addition a glossary (mainly medical terms) and a list of symbols with their explanations are included.

The authors have provided a good balance of textual material, structural formulas, reactions and tables. All of the chapters except four are followed by a series of questions. The index of 59 pages is very complete but could still be improved by a more liberal use of heavy type to indicate the most important page reference or references when several are given for a single subject.

The authors make frequent use of electronic formulas to explain the mechanism of reactions. The I. U. C. system of nomenclature is explained and is stressed throughout. In keeping with the growing importance of the subject, particular attention is paid to physiologically active compounds. In fact, with a few notable exceptions, *e. g.*, amaranth and diphenylhydantoin, most of the organic compounds of the current revision of the United States Pharmacopœia are discussed from a chemical standpoint, and the pharmacological activity of many is given in an abstract form.

These and other outstanding features of the book will recommend it as a text and as a reference work. It would be especially useful as a text in those schools where organic chemistry and the chemistry of organic medicinal products are taught as a combined course.

The typography is above the average; the paper is of good quality; and the binding is of dark blue vermin-proof cloth.—W. T. SUMERFORD.